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BIOLOGICAL BULLETIN.

AN AMERICAN CERAPACHYS, WITH REMARKS ON THE AFFINITIES OF THE CERAPACHYINÆ.[1]

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The singular ant described in the following pages was discovered by Miss Augusta Rucker within the city limits of Austin, Texas, on May 11 of the current year. Impressed by the peculiar, elongate, and segmentally constricted body of the insect, Miss Rucker fortunately secured nearly all the individuals in the nest, including the apterous queen. The most superficial study showed that the insect could belong to none of the described North or South American genera of Formicidæ. A note from Professor Emery, to whom a few of the specimens were sent, and comparison with some rare ants generously sent me by that gentleman and by Dr. Gustav Mayr, made it perfectly evident that the new form must be assigned to the interesting genus *Cerapachys* (subgenus *Parasyscia*), representatives of which were hitherto known only from the Old World (Africa, Asia and Papuasia).

The *Cerapachys* nest was found about six inches below the surface soil under a layer of large flakes of limestone in the shade of some hackberry trees growing on the banks of Waller Creek. At first only six workers and the queen were taken, but on the following day Miss Rucker and myself succeeded in unearthing four more workers on the same spot. The whole nest could not have contained much more than a dozen specimens. Unfortunately there were no larvæ or pupæ. When first seen the *Cerapachys* were engaged in a battle with some much smaller subterranean ants (*Ponera trigona* var. *opacior*) which seemed to be invading the nest. The new species was slow in its move-

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 37.

ments as would be expected in creatures with such long bodies and short legs. They did not "feign death" when roughly handled, though some of them, when held in the forceps, remained motionless in the attitude probably assumed when they are being deported by their sister workers. While creeping about the ants carried their very robust antennæ in a peculiar manner. The scape was held erect or inclined forwards, but hardly in a horizontal or lateral position as in other ants, while the funicle could be folded down onto the front surface of the scape a little obliquely to the side. The habits, so far as these could be inferred from the little I saw of the ants in a living condition, recalled those of *Stigmatomma pallipes* described in a former paper ('00). As the workers are quite blind it seems probable that the species leads a subterranean life, seeking its prey in the soil or under the dead leaves. This seems also to be indicated by the depth at which these small insects were found. The very robust antennæ and the beautifully developed stridulatory apparatus, which occupies the whole of the large membrane between the postpetiolar and first gastric segment, indicate that the senses of contact-odor and hearing are highly developed and may adequately compensate for the absence of visual organs.

On the 22d of May, Mr. C. T. Brues found a solitary worker of the same *Cerapachys* under a stone at Pease Park, about two miles from the locality in which the species was first taken. That the insect is extremely rare is shown by the fact that it had not been found before during three years of careful collecting in the vicinity of Austin.

CERAPACHYS (PARASYSCIA) AUGUSTÆ, sp. nov.

Worker (Fig. 1).—Length 2.5–3.5 mm. Head longer than broad, marginate and broadly excised behind and produced posteroinferiorly to form two acute, somewhat divergent angles, so that the head resembles in shape that of *Eciton schmitti* Emery. These posterior angles are continued downwards on either side as a fold which meets its fellow from the opposite side on the lower posterior surface of the head. Sides of head faintly and evenly convex; eyes entirely absent; lateral carinæ well-developed; frontal carinæ high, projecting, closely approximated, extending a short distance back between the antennal foveæ and ending on either side in a distinct tooth just in front of the rather pronounced frontal depression. Mandibles triangular, curved downwards at their tips, with

distinctly crenated edges to their blades. Antennal scape somewhat more than half the length of the head exclusive of the mandibles, rapidly incrassated towards its apex, which is provided with a deep concavity on the anterior lateral surface for the insertion of the funicle; funicle 10-jointed; first joint longer than broad, almost concealed in the concavity of the scape; joints 2–9 distinctly broader than long, gradually increasing in size distally, terminal joint very large, glandiform; constituting a club which is as long as the five preceding joints of the funicle. Thorax cylindrical, fully two and one half times as long as broad, oblong when seen from above, dorsal surface flattened, mesoëpinotal suture hardly indicated by a faint constriction. Posterior surface of epinotum abruptly declivous, carinate on either side and with an indistinct tooth above. Petiole subcuboidal, a little longer than broad, with flat dorsal surface; lower surface produced anteriorly into a large, compressed, plowshare-like tooth. Postpetiole flattened dorsally, one and a half times as long as the petiole; when seen from above its anterior margin is hardly broader than the petiole, but its posterior border is half again as wide; its lower surface is convex and projects forward a little in front of the anterior dorsal border. Stridu-

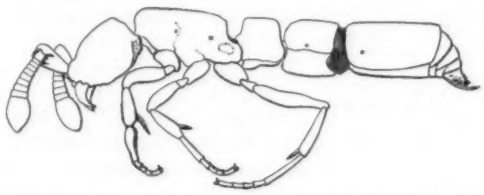

FIG. 1. *Cerapachys* (*Parasyscia*) *augustæ* n. sp. Worker.

latory organ highly developed, occupying the whole of the intersegmental membrane between the postpetiole and gaster. First gastric segment cylindrical, flattened on its dorsal surface, fully one and a half times as long as the postpetiole, slightly wider behind than in front. Remaining gastric segments very short, forming a rapidly declivous termination to the abdomen; second, third and fourth gastric segments of about equal length, tergite of the fifth segment triangular, covered with small but distinct spines on its lateral and posterior border. Sting thick, exserted. Legs rather short, all the tibiæ furnished with pectinate spurs.

Surface of body shining, except the head, which is subopaque. Mandibles finely and indistinctly striated. Head covered with large, close-set, umbilicate foveolæ except on the folds of the posterior angles which are coarsely coriaceous. Whole thorax covered with umbilicate foveolæ like those on the head. On the petiole and postpetiole the foveolæ are as large as those on the head and thorax but less densely aggregated; on the first gastric segment the foveolæ are distinctly smaller and much further apart.

Whole body covered with long, suberect, golden yellow hairs, which on the head, thorax and abdomen, arise from the umbilicate centers of the foveolæ. Hairs on the terminal antennal joint very short and dense, contrasting with the longer hairs on the scape and short joints of the funicle.

Color red, edges of mandibles, clypeus, anterior border and posterior angles of head, the funicle with the exception of the terminal joint, the articulations of the thorax, legs and abdomen and the tip of the latter blackish. Legs and terminal antennal joint slightly more yellowish than the remainder of the body.

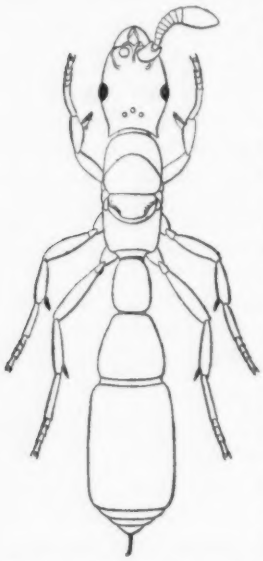

FIG. 2. *Cerapachys* (*Parasyscia*) *augustæ* n. sp. Apterous female.

Female (Fig. 2).—Length 3.75 mm.

Eyes moderately large, convex, situated in the middle of the lateral surface of the head, which is shaped like that of the worker. Ocelli well developed, not lying at the corners of an equilateral, but of an isosceles triangle with a long base. Prothorax large, scutum of mesonotum well developed, dorsally flattened, without parapsidal sutures; tegulæ large, elliptical; no paraptera between the scutum and the well-developed, flattened scutellum, metanotum narrow but distinct; epinotum large, shaped like that of the worker. On the pleural surface the mesothoracic epimerite and episternum are distinct but these elements in the metapleuræ are more obscurely separated. There is nothing to show that the thorax has ever borne wings. Petiole longer than broad, postpetiole almost twice as broad as the petiole, its posterior border nearly coëxtensive with the edge of the first gastric segment, which is both broader and longer than this segment in the worker. Terminal gastric segments and sting in all respects like those of the worker.

The sutures of the thoracic dorsum are blackened; otherwise the female is like the worker in coloration, sculpture and pilosity.

As *Cerapachys augustæ* has 11-jointed antennæ it must be placed in Emery's subgenus *Parasyscia*. Emery ('01[a] and '01[b]) has recently published a revision of the ants of the subgenus *Cerapachys* and the allied genera which he groups together as Cerapachyinæ, a supertribal division comprising the following tribes, genera and subgenera.

Tribe 1. Acanthostichii—with the single South American genus *Acanthostichus* Mayr.

Tribe 2. Cerapachyi (Africa, Asia, Australasia and Texas!).

Genus *Cerapachys* Smith (antennæ with a one-jointed club).

Subgenus *Cerapachys* (antennæ 12-jointed).

Subgenus *Parasyscia* Emery (antennæ 11-jointed).

Subgenus *Oöceræa* Roger (antennæ 10-jointed).

Subgenus *Syscia* Roger (antennæ 9-jointed; first gastric segment but little longer than the postpetiole).

Subgenus *Cysias* Emery (antennæ 9-jointed; first gastric segment much longer than the postpetiole).

Genus *Phyracaces* Emery (terminal antennal joint not forming a club — Madagascar, Africa, Borneo).

Genus *Lioponera* Mayr (2–3 terminal joints of antenna forming a club — India, Sumatra).

Genus *Sphinctomyrmex* Mayr (abdomen constricted behind each segment — Brazil).

Tribe 3. Cylindromyrmii.

Genus *Cylindromyrmex* Mayr (antennæ 12-jointed; South America).

Genus *Simopone* Forel (antennæ 11-jointed; Madagascar).

The occurrence in America of a representative of the largest and most diversified genus of the Cerapachyinæ is of some interest in view of the fact that this group is the most archaic and generalized of existing Formicidæ. It is, in fact, the group from which Emery and Forel would derive both the Dorylinæ and Ponerinæ, themselves very primitive subfamilies of ants. Inasmuch as the three remaining subfamilies (Myrmicinæ, Dolichoderinæ and Camponotinæ) are derivable from Ponerine forms, it is evident that the Cerapachyinæ must constitute a group of high phyletic significance. Emery ('95) has even gone a step further and pointed out the close resemblance of the Cerapachyi to the Mutillidæ, especially to forms like *Apterogyna*, which have a very ant-like pedicel to the abdomen and resemble the ants in many other particulars. I have copied his figure of *Apterogyna* (Fig. 5) for the sake of showing the close resemblance of this primitive Mutillid to certain species of *Cerapachys*, *e. g.*, *C. peringueyi* (Fig. 3).

While Emery and Forel agree in regarding the Cerapachyinæ as the most primitive of Formicidæ, they hold very different

opinions concerning the subfamily to which the group should be assigned. Emery ('95, '01[a]), who emphasizes morphological characters, regards the Cerapachyinæ as veritable Dorylinæ, while Forel ('99, '01), who is inclined to lay considerable stress on ethological characters, maintains that these ants are true Ponerinæ. Accordingly Emery includes the Cerapachyinæ and Dorylinæ (*sens. str.*) as two coördinate groups under the sub-

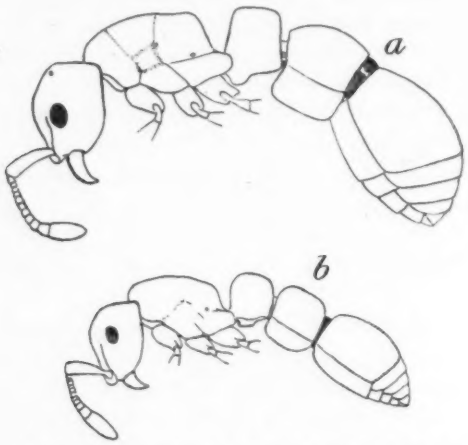

FIG. 3. *Cerapachys peringueyi* Emery. *a*, female (apterous); *b*, worker. After Emery.

family Dorylinæ, whereas Forel would regard the three tribes Acanthostichii, Cerapachyi and Cylindromyrmii as so many tribes of Ponerinæ.

Emery's position may be stated as follows: Almost nothing is known concerning the habits of the Cerapachyinæ and mere ethological inferences cannot help us in deciding the question of affinities. The female of *Acanthostichus* (see Fig. 4, *a*) is wingless and decidedly *Dichthadia*-form ("bâtie sur le type *Dichthadia* à peine mitigé"), like the females of *Dorylus* and *Eciton*. The males of the Cerapachyinæ have no cerci and have retractile copulatory organs like the Dorylinæ, whereas the cerci are present and the genitalia more or less exserted in the male Ponerinæ. In general, comparatively little value can be attached to the con-

ditions of the pedicel in the taxonomy of ants, so that we should not emphasize the Ponerine-like petiole and postpetiole of the Cerapachyinæ, especially as all sorts of pedicels are found among the different genera of this group from that of *Acanthostichus*, which is like *Amblyopone*, to that of *Oöceræa*, which recalls the conditions seen in the Myrmicinæ. The larvæ of the Cera-

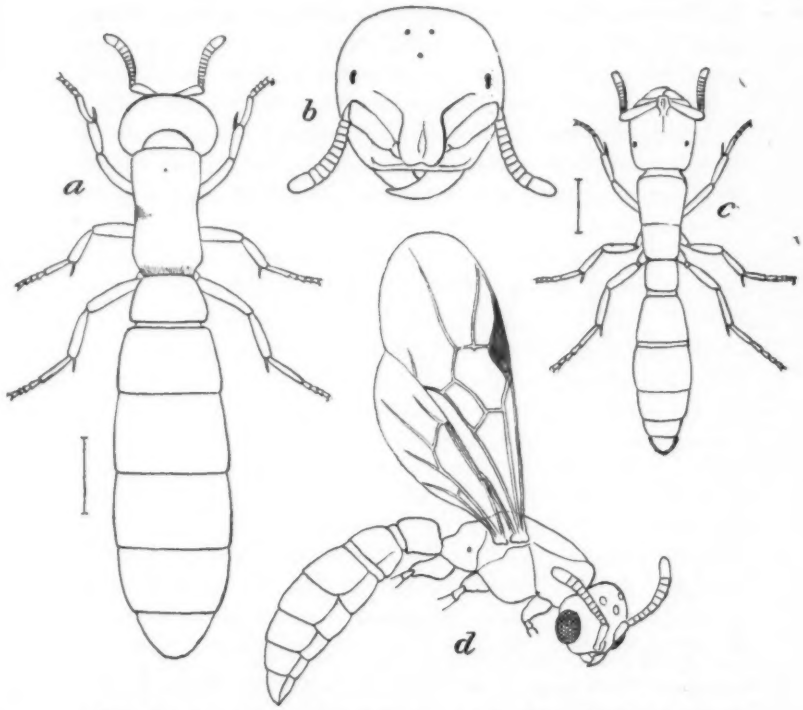

FIG. 4. *a*, *Acanthostichus quadratus*, ♀ ; *b*, head of same from front ; *c*, large worker (same magnification as *a*) ; *d*, *Acanthostichus fuscipennis*, ♂. After Emery.

pachyinæ are unknown, but as non-tuberculate larvæ, somewhat resembling those of *Eciton*, occur in some distinctively Ponerine genera (*Stigmatomma* and *Ectatomma*) it is probable that the larval characters would have little weight in solving the problem under consideration.

Forel, on the other hand, advances the following reasons for regarding the Cerapachyinæ as true Ponerinæ. While they undoubtedly exhibit traits which ally them with the Dorylinæ, their habitus is, nevertheless, decidedly Ponerine. The little that is known of their habits certainly indicates that they live in small, stationary colonies like the Ponerinæ, instead of populous, nomadic colonies like the Dorylinæ. The queens, moreover, are so nearly of the same size as the workers as to preclude anything like the great fecundity of the queens of *Dorylus* and *Eciton*. The Cerapachyinæ, too, have short legs of such a structure as to indicate a slow gait and more sedentary habits. The workers of the Cerapachyinæ have ordinary facetted eyes, whereas those of the Dorylinæ are absent or ocelliform, while the atrophied eyes of the Ponerinæ have a very different structure.[1] The conditions of the

FIG. 5. *Apterogyna olivieri*. Female, after Emery.

pedicel in the males, females and workers of the Cerapachyinæ are correlated as they are in the Ponerinæ, and do not exhibit the differences seen in the Dorylinæ between the worker on the one hand and the male and female on the other. The wingless con-

[1] The distinction to which Forel calls attention is worthy of histological study. I am inclined to think, however, that it may be a distinction without a difference. I have recently sectioned a number of pupæ of *Eciton schmitti* and find that the ocelliform lateral eyes are really very much atrophied compound eyes, too much atrophied, in fact, to be at all functional as visual organs. *The retinal hypodermis, which is somewhat thickened under the convex lens, shows indistinct but unmistakable traces of ommatidia. The optic nerve is very short and not connected with the brain. It ends freely in a blunt point a short distance from the ommatidial layer. This is interesting as proving that the visual fibers must arise in the retina and grow towards the brain and not in the reverse direction from cells in that portion of the brain known as the optic ganglion.* If there is a distinction between the abortive eyes of the Dorylinæ and Ponerinæ it would seem to be that in the former subfamily the ommatidia disappear both by fusion with one another and by reduction in number, while in the latter the number of ommatidia is gradually reduced without fusion.

dition of the female Dorylinæ is not of so much value in view of the fact that some true Ponerinæ (*Leptogenys*, *e. g.*) have wingless ergatoid queens. The characters drawn from the male genitalia are not of themselves sufficiently important to determine the allocation of the Cerapachyinæ in a particular subfamily.

My own observations on the living *Cerapachys augustæ*, though very fragmentary, incline me to accept Forel's views and to regard the Cerapachyinæ as true Ponerinæ. There was certainly nothing in the habits of the insects to remind me of the Doryline ants notwithstanding their striking morphological resemblance, especially in the shape of the head, and the blindness of the workers, to certain species of *Eciton* (*E. schmitti* Emery, *E. sumichrasti* Norton, *E. wheeleri* Emery). The small colony, with its queen so like the workers in size and structure, and the slow movements of the insects, all very closely resemble the conditions found in many lowly organized Ponerinæ, *e. g.*, *Stigmatomma*, and I may add also, *Proceratium* and *Sysphincta* to judge from the account (*in litteris*) of my friend, Rev. P. J. Schmitt, O.S.B., who has been so fortunate as to observe living colonies of these rare ants. Moreover, the fact that specimens of Cerapachyinæ are rare in collections would seem to show that the nests of these insects are not at all populous. It is safe to say that if *C. augustæ* were like the timid *Ecitons* in its habits, it would have been impossible to find a remnant of the colony on the day following the ransacking of the nest. Morphologically the Ponerinæ are certainly a very heterogeneous assemblage of forms, but this is merely what we should expect to find in so ancient and extensive a group. The addition of the tribes of the Cerapachyinæ to this subfamily would increase but little the already existing heterogeneity, since these ants are closely related to forms like *Proceratium* and *Sysphincta* which Emery formerly regarded as Dorylinæ but has since placed with the Ponerinæ. When the Cerapachyinæ are included with the Ponerinæ, the Dorylinæ become a more homogeneous subfamily, while the Ponerinæ represent the diversified and often peculiarly specialized survivors of the ancient stock from which all the other subfamilies of ants have been descended.

It is an interesting and probably significant fact that all the

various forms of females to be met with among the Formicidæ are already foreshadowed in the small and very primitive group of the Cerapachyinæ. Though we have no knowledge of the females of several of the genera, we may recognize no less than four different female forms:

1. The female of *Acanthostichus*, which, as Emery has shown ('95), is decidedly *Dichthadia*-like, *i. e.*, unmistakably like the huge blind and wingless females of *Dorylus* and *Eciton*. This female is considerably larger than the largest workers of the colony as shown in Emery's figures of *A. quadratus* which I have reproduced in outline (Fig. 4, *a*, *b* and *c*).

2. Normal winged females like those of most genera of Formicidæ but more similar to the workers in size and structure. These females are known to occur in the genera *Lioponera*, *Cerapachys* and *Sphinctomyrmex*.

3. The female of *Cerapachys peringueyi* from South Africa (Fig. 3, *a*). According to Emery ('95) this form is wingless and not much larger than the worker (Fig. 3, *b*), which it closely resembles in structure. It may be designated as an ergatoid female and is not unlike the ergatoids occasionally found in species of *Ponera* (*P. coarctata*, *P. opaciceps*, etc.).

4. The female represented by the above described *Cerapachys augustæ* from Texas. This form is wingless but in thoracic structure resembles the winged females of the Ponerinæ in general. It is but little larger than the largest workers though possessing well-developed eyes and ocelli.

This "morphological restlessness" in the structure of the females of so small a group of genera as the Cerapachyinae is, perhaps, significant as the phyletic source to which the different female forms of all the subfamilies of ants are to be traced. We may look upon the *Dichthadia*-like queens of the Dorylinæ as a further development of the conditions exhibited by *Acanthostichus*, and the ergatoids, which crop out sporadically among the Ponerinæ and the higher subfamilies, may, perhaps, be regarded as cases of reversion to females of the type of *Cerapachys peringueyi* and the Mutillidæ (*Apterogyna*, *e. g.*). The pseudogynic forms of the more specialized ants (*Formica*, *Camponotus*) resemble the female of *C. augustæ*. Finally, the winged females

of *Lioponera*, *Sphinctomyrmex*, etc., like the normal winged females of the majority of the Ponerinæ, lead very naturally to the conditions seen in the more modified winged females of the Myrmicinæ, Dolichoderinæ and Camponotinæ.

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ROCKFORD, ILLINOIS, July 6, 1902.

STUDIES ON THE LIFE-HISTORY OF PROTOZOA. III, THE SIX HUNDRED AND TWENTIETH GENERATION OF PARAMŒCIUM CAUDATUM.[1]

GARY N. CALKINS.

Over 65 years ago, Ehrenberg came to the conclusion that the Protozoa are potentially immortal. Having all of the necessary organs and losing nothing in reproduction, there was no reason at all, he argued, why they can not live indefinitely. Shortly after this, Dujardin published a work in which he stated that Protozoa have periods of activity alternating with periods of rest, and Ehrenberg's view was vigorously contested.

Among contemporaneous biologists, the matter has been taken up, on the one hand, by Weismann and his followers, on the other by Maupas and those who see in Maupas' work the best weapon with which to combat Weismann. The matter at issue involves an interesting question, *viz.*, will protoplasm having every opportunity to exercise its various activities, and without being specialized through differentiation to the performance of limited functions, continue to live indefinitely, or will it gradually become exhausted and die as do the multicellular animals? Weismann, following the line of reasoning pointed out by Ehrenberg, maintains the former point of view, while Maupas, on the other hand, upholds the latter and argues, that, as the race becomes older, the vitality becomes more and more reduced, until finally, the Protozoön, like the Metazoön, dies from old age. Both writers have had to take into consideration the factor of conjugation with the accompanying phenomena which were almost entirely unknown to the earlier controversialists. With Weismann, conjugation is merely one of the vital functions of protoplasm, to be considered in the same light as feeding or excreting and not in any way interfering with the theory of immortality. Maupas

[1] A lecture delivered at the Marine Biological Laboratory, Woods Holl, Mass., July 28, 1902.

argues that, like Metazoa, the race would show unmistakable signs of senile degeneration and would die of old age were it not for conjugation which, in some unknown way, restores the vitality and ensures a new cycle of generations. According to Maupas, therefore, it would seem that the protoplasm of the unicellular organisms is not capable of the perpetual exercise of the ordinary vital functions. His conclusions lead to the assumption that, after conjugation, the individuals have a certain potential of vitality which is gradually used up in the course of a more or less definite number of generations, and which can be restored again and again by conjugation.

The general theories involved in this discussion seem to me important enough to warrant further experimental tests, and with this in mind, I started the culture of two different lines of *Paramœcium caudatum*, on the 1st of February, 1901, and these have been under continual observation ever since. One of the lines died out last month, in the 570th generation, the other is still living in the 665th generation.

One definite aim at the outset was to ascertain whether a given form under culture would pass through a more or less clearly-marked cycle of activity to die ultimately of old age as Maupas maintained. Another aim was to find out whether there is a well-marked degeneration of different parts of the cell as age advances. These aims merely called for the repetition of Maupas' work in the case of one organism and were interesting enough in themselves. There was still another aim, however, which lent added zest to the work and made every stage one of fascination. This aim was to find out, if possible, the significance of conjugation and why it is that vitality is renewed by this means. It seemed to me that if Professor Loeb could induce artificial fertilization in the echinoderm egg, it should be possible to induce artificial rejuvenescence in *Paramœcium*. The first definite problem that suggested itself was the discovery of some substitute for conjugation that could be used when the periods of physiological depression were imminent. The experiments have justified the assumption that such substitutes would prove successful, for I have been able to carry these minute organisms, not through merely one period of so-called degeneration, but

through five such periods, and have been able to rejuvenate them "parthenogenetically" five successive times. I feel that I am now in the position to confirm the early observation of Dujardin that, in *Paramœcium* at least, there are more or less regular periods of vigor and depression which alternate with each other in fairly regular succession. At each period of depression, the organisms, had they not been stimulated in some way, would have died, thus showing that they were physiologically worn out. The subtitle of this paper is taken from one of the periods of depression when the last survivors of a long line were nearly lost.

The methods employed in the investigation are practically the same as those used by Maupas. The infusoria are kept in cells in depression slides filled with a food medium of hay-infusion. The original *Paramœcium* of the A series was taken from the pond water of Van Cortland Park, New York, and transferred to one of these cells. In two days it had divided three times and of the resulting eight individuals four were isolated in the same way as the first had been, while the remainder were set aside as "stock." The four lines thus started have been maintained throughout and give a better basis for the study of vitality of the race than one line could possibly have given. A second series—B—was started at the same time with an individual *Paramœcium* from Fort Lee, New Jersey, and four lines of this series were likewise carried on with the A series. Individuals are isolated every one or two generations and the records are kept in the form of a table showing the number of generations since the last isolation and the total number since the beginning.

The division-rate is taken as the measure of vitality, for it represents the rate of metabolism, growth and reproduction. A better index of the general vitality could not be found and while fairly constant from day to day, its fluctuations mark out clearly the periods of vigor and depression. The variations of the rate are difficult to follow when the daily records alone are considered and the cultures had been under way for ten months before a method was discovered of representing it graphically. When the daily rate of division is plotted the fluctuations of the curve, due partly to varying temperature and the food conditions, are perplexing and it is extremely difficult to compare the con-

dition of the general vitality at different periods by this means. Good results were finally obtained by averaging the number of divisions per day for definite periods, and plotting the averages. By this method of illustration the condition of the general vitality at any period desired can be seen at a glance. (See Fig. 1.)

The division-rate, as represented by such a curve, continued without noticeable variations from the beginning of the experiments until the latter part of the following July. About the 20th of that month the organisms began to die off at an alarming rate, indicating that a period of depression had apparently set in, or "degeneration" in the Maupas sense. Now was the time, if at all, to experiment with substitutes for conjugation, and in conformity with my original plan, the first trials were made with a change of diet. The organisms had been living steadily on the bacteria that develop in hay-infusion (*Bacillus subtilis* mainly), and various substitutes were now tried. Vegetable infusions of different kinds gave no improvement but meat infusions proved successful. The first experiment with the latter was with teazed liver which was added to the usual hay-infusion. The result was very gratifying for the organisms began immediately to grow and to divide, the rate of division rising from 5 to 9 divisions in successive 10-day periods. The effect of the liver was not lasting, however, and in the following 10-day period, the rate fell off to one division in five days or two-tenths of a division per day. During this time the organisms had not been continuously on liver treatment but had been transferred to the usual hay-infusion diet after 48 hours in the liver medium. At the end of the first 10-day period in August the cultures were again dying off, this time more rapidly than before and the renewed use of teazed liver had absolutely no effect. One thing after another was now tried without success, the organisms meanwhile growing weaker and weaker. Finally they were transferred to the clear extract of lean beef made by boiling fine pieces of beef in tap water. The effect of this medium was interesting, for, although it restored the weakened vitality, there was no rapid increase in the rate of division as when first treated with the teazed liver. The infusoria were, however, now large and vigorous and did not die unless transferred from the beef medium to the usual hay infusion. When this was attempted,

they would become abnormally active and would finally die. The division-rate gradually increased during the month of August until, in the last ten days, they averaged six generations. Finally, in September, the attempts to get them back on the old diet of hay-infusion were successful, and then the division-rate went up at once to twelve times in ten days, and a month later, they were dividing at the rate of fifty times a month.

This experience showed that "rejuvenescence" in the Maupas sense, can be brought about without the aid of conjugation. Had it not been for the extract of beef, or some other equally beneficial substance, all of the organisms would have died, and, like Maupas,[1] Joukowsky[2] and Simpson,[3] I should have assumed that their possibilities of continued living had been exhausted and that they had died from "old age" in the 198th generation.

From November 1st to December 10th the curve shows that the division-rate and the general vitality gradually decreased, and by the middle of December, a similar experience to that of mid-summer threatened the cultures. Profiting by that experience I took the precaution of giving some of the individuals beef-extract before they got too weak, while others were continued on the hay-infusion. All of the latter died within a few days. Those that had been fed with beef-extract for 48 hours continued to live when put back in the hay-infusion, and they continued through another cycle of almost three months without treatment again with the beef.

From the middle of December on, the cultures were continued under slightly different treatment. Two of the four lines of As (A–1 and A–2) and two of the four lines of Bs (B–1 and B–2), were treated once per week for 24 hours with beef extract. This treatment was continued until the middle of March, when they were given hay-infusion without further variation. The other lines of As and Bs, *viz.*, A–3, A–4, and B–3, and B–4, were treated in December with the beef-extract and then kept on hay-infusion without further change. By this treatment it was possible to compare the general vitality under conditions of a more or less

[1] Maupas, E., *Archives d. Zoöl. expér. e. gén.* (2), VI. and VII., 1888, 1889.
[2] Joukowsky, D., *Verh. nat. Med. Ver. Heidelberg*, 1898, Bd. XXVI.
[3] Simpson, J. Y., *Proc. Roy. Soc. Edinburgh*, 1901.

regular stimulus, with the general vitality under conditions of an initial stimulus. The history of these experiments is shown graphically in the diagram shown on page 198.

On the first of January, all were in fairly good condition and the division-rate was about the same for all. All showed a decline in the latter part of February, and the rate of division rose for all of them in March. At this time there was a sudden warm period in which the temperature for that ten-day period increased 3 degrees on the average and this apparently acted as a mild stimulus. From this time on, however, the division-rate of the hay-fed individuals began to fall, and as soon as this fact was noticed, the beef treatment of the others was stopped. The hay-fed individuals did not recover their vitality and their division-rate continued to fall until finally stock and all died, apparently worn out. The beef-fed individuals, on the other hand, continued their high rate of division throughout the period of decline of their sister-cells, and did not show signs of diminished vitality until the first period in June. At this time, however, the rate fell very rapidly and the depression was clearly apparent in the daily records (See Fig. 1). The organisms at this time had not had beef-extract since March and they were accordingly transferred to it now with perfect confidence on my part that it would again prove to be the "elixir of life" for the *Paramœcium. This time, however, it failed to give the looked-for result, and the animals continued to die at a rapid rate.* Their appearance indicated that their trouble was not the same as in the previous periods of depression. Hitherto at such times the endoplasm appeared black under the microscope from the large numbers of gastric vacuoles with their food contents unchanged. At this time, on the other hand, the endoplasm was clear and free from such food masses, but the appearance of the protoplasm was singularly granular and different from that of a healthy individual. All changes that had proved beneficial before were now tried. These included not only liver, various vegetable infusions and the like, but salts of various kinds. Nothing seemed suitable for the organisms and they died off at a faster rate than ever. *When the last of the B series, stock and all, died in the 570th generation (June 16), and the A series became reduced to only 6 individuals in the 620th*

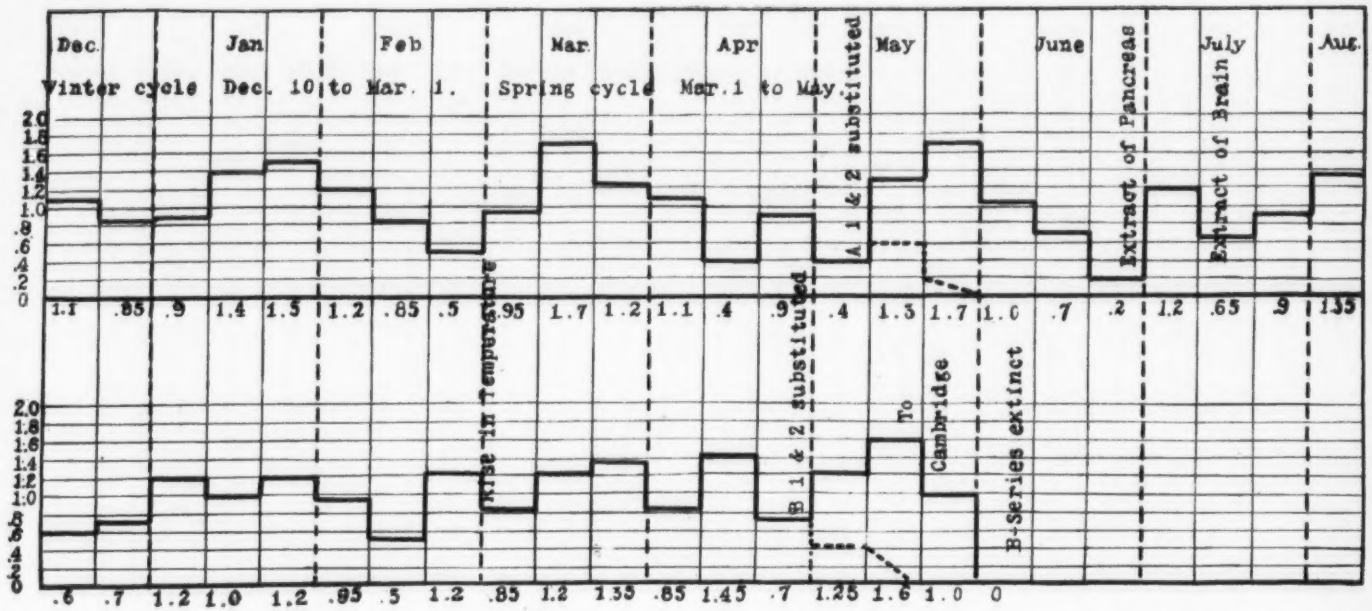
Dec.
Jan
Feb
Mar.
Apr
May
June
July
Aug.
Winter cycle Dec. 10 to Mar. 1.
Spring cycle Mar. 1 to May.
A 1 & 2 substituted
Extract of Pancreas
Extract of Brain
Rise in temperature
B 1 & 2 substituted
To Cambridge
B-Series extinct
1.1 .85 .9 1.4 1.5 1.2 .85 .5 .95 1.7 1.2 1.1 .4 .9 .4 1.3 1.7 1.0 .7 .2 1.2 .65 .9 1.35
.6 .7 1.2 1.0 1.2 .85 .5 1.2 .85 1.2 1.35 .85 1.45 .7 1.25 1.6 1.0 0

FIG. 1. Curve showing the history of the cultures from December 10, 1901, to August 10, 1902. The upper curve represents the A-series, the lower one, the B-series. In the former, the daily division-rates for A–3 and A–4 are averaged and these again are averaged for 10-day periods. In the latter, B–3 and B–4 are averaged in the same way. A–3, A–4, B–3 and B–4 were given beef extract in December and were then continued on hay-infusion until all four lines became extinct in May. At this time it became necessary to substitute A–1, A–2, B–1 and B–2 for the weakening sister lines whose further history is indicated by the dotted lines. The ones that were substituted had been fed once per week for 24-hour periods with beef-extract, but this treatment was stopped in March and they were given hay-infusion from that time on. The cycles thus overlap, for the beef-fed lines in March were in the s.. stage, as regards stimulation, as the hay-fed ones were in December. The B-series became extinct in June; the A-series were saved by change of diet. Those that were "rejuvenated" by extract of brain were substituted for those that were treated with extract of pancreas as soon as it was seen that the latter did not have a permanent effect.

generation (June 27th) it looked as though the cultures were about to come to an end. The unusual appearance of the organisms made me think that the trouble was not with the digestion of food, but with its assimilation, and with this clue in mind I made extracts of sheep's brain and sheep's pancreas. Three of the six remaining individuals were put into the brain-extract and three into the extract of pancreas. The latter worked best and the 621st generation appeared the next day. The former, while giving slower results, produced healthy and normal organisms, and in three days there were more than 30 individuals, and enough to experiment with. After 48 hours' treatment with each of these extracts the organisms of the regular series were transferred to the usual hay-infusion. Since then the division-rate has been fairly constant, and, as stated at the beginning of this paper, they are to-day (July 28th) in the 665th generation.

The effect of the two extracts was very interesting. The individuals that were treated with pancreas had been in N/25 magnesium chloride for thirty minutes on the sixth day of May. Those that were treated with the brain-extract had been in N/1250 potassium phosphate for thirty minutes on the eighth of June. The division-rate of the former, notwithstanding the quicker start, soon fell behind that of the latter, and to-day the only ones alive are the descendants of those that had been fed with the brain-extract.

The interpretation of these facts involves the interpretation of many of the deepest problems of biology. If we could point out their significance, it would mean that we are in a position to throw light upon the ultimate secrets of vitality; secrets which have taxed the ingenuity of philosophers of all ages, and which lie at the bottom of modern biological research. At the present time the question cannot be answered, and it seems mere presumption to attempt an explanation. Nevertheless, there are sufficient data to warrant a tentative interpretation even though it is only a vague, shadowy impression of what may actually go on in living protoplasm.

The bacteria in the hay-infusion constitute the normal food of the Paramœcidæ. Of these, *Bacillis subtilis*, is, probably, the only one left alive after the infusion is raised to the boiling point, and

this organism, therefore, forms the staple article of diet for *Paramœcium* in culture. In the body of the infusorian the bacteria are collected in the usual gastric vacuoles where the first stages, at least, of digestion are carried on. No one has identified the proteolytic ferment which is active in this process. The work of Meissner, LeDantec, Fabre-Domergue, Greenwood and others has shown that something analogous to pepsin plays the most important rôle in the process, but nothing definite concerning the ferment is known. In an allied group, however, the Rhizopoda, some work has recently been done which may throw light upon the digestive processes of the Infusoria. H. Mouton,[1] working in Metschnikoff's laboratory, has succeeded in extracting the proteolytic ferment from a bacillus-eating *Amœba*. Tests of various kinds showed that the ferment is more like trypsin in its action than like pepsin, and Mouton comes to the conclusion that it is intermediate between the two. As the foods of this *Amœba* and of *Paramœcium* are probably similar in proteid composition, it is not improbable that the proteolytic ferments are much alike.

Whether or not the proteolytic ferment in *Paramœcium* is the same as that in *Amœba*, there is no doubt that some such active agent is operative in the digestive processes of our infusorian. Furthermore, there is a certain amount of evidence to indicate that it is this ferment-forming power that becomes weakened in the protoplasm of *Paramœcium* as the race grows older. Physiological "degeneration" is not due, as Maupas supposed, to the loss of some of the necessary organs of the cell through degeneration, nor is it due to inability to take food, for, when in the depressed condition, the *Paramœcium* still maintains a current of bacteria into the mouth-opening, and food vacuoles are still formed. Frequently when in this condition the organisms appear black because of the undigested food balls, a fact which indicates that it is the digestive function that wears out. In this connection it is interesting to note the effect of dilute alcohol on the general metabolism of *Paramœcium*. Mr. C. C. Lieb, work-

[1] H. Mouton. Recherches sur la digestion chez les Amibes et sur leur diastase intracellulaire. Thesis presented at the Faculté de sciences de Paris, 1902. Reprint pp. 1–60.

ing with *Paramœcium* from these cultures, found that alcohol will increase the division-rate above the control, by about 33 per cent. Under the action of the alcohol the organisms are always lively and appear transparent as though all food were rapidly digested. The result is interpreted as a stimulus to the digestive processes effected by the alcohol.[1] In the period of depression which is represented by the 620th generation, alcohol brought about no stimulus and the organisms died as rapidly as the ones not thus treated.

As the age of the race increases, it is conceivable that something in the chemical make-up of the protoplasm becomes depleted, something which cannot be replaced from the constituents of the ordinary food medium. With the change of diet, however, new substances are introduced into the protoplasm and renewed activity results. Thus with the change from hay-infusion to beef-extract, chemical substances are introduced into the protoplasm which could not be obtained from the hay-infusion. In the New York water there are certain chlorides, nitrates, etc., all in extremely minute quantities and these are present of course in the hay-infusion. They are also present in the beef-extract which is made with the same water, but in addition to these salts there are certain extractives from the beef such as potassium phosphate and chloride, lactic acid and lactates, etc., substances of quite a different character from those of the ordinary medium. Added to the fact of a different chemical medium is the fact of a difference in osmotic pressure, for the extract is more dense than the hay-infusion. The extract does not have a direct stimulating effect upon the digestive processes and upon division, for, while the organisms are immersed in it, there is a very slow division rate; when transferred again to the hay-infusion, however, they divide more rapidly than before. From this it appears that the extract is not a food in the ordinary sense but that it is a stimulant acting through the salts dissolved in it.

These results justify, I believe, the conclusion that, as the potential of vitality becomes reduced, something in the chemical

[1] Cf. Calkins and Lieb. Studies on the Life-History of Protozoa. II., The Effect of Stimuli on the Life-Cycle of *Paramœcium caudatum*. *Arch. f. Protistenk.* I., 3, 1902.

composition of *Paramœcium* wears out or disappears so that the ferment-forming activity is lessened. A change of diet restores the power. The restoration may be effected, not only by the extract of beef, but by clear chemicals as well. Thus potassium phosphate, potassium chloride, sodium and magnesium chlorides, have all given positive results in this direction, and it is probable that these substances contain the elements needed by the weakened protoplasm. We are justified, therefore, in regarding the artificial rejuvenation of *Paramœcium* as a phenomenon of the same order as the chemical fertilization of the egg.[1]

The general problem may be attacked from another point of view. What is it that conjugation does to renew the potential of vitality? The early conception of Butschli's that conjugation brings about a renewal of youth or a *Verjungung* of the participating organisms has been repeatedly confirmed by subsequent observers. A very significant fact came to light in the course of these experiments and one which seems to show that conjugation, like the action of the beef-extract, involves certain chemical phenomena. It will be recalled that Maupas maintained that endogamous conjugation is fruitless and the reason given was that, as the cultures grow older, the micronucleus degenerates and disappears. Maupas did not keep *Paramœcium* in culture for more than a few weeks, but the period was long enough for him to feel justified in assuming that this form is similar to the other forms examined by him. He declared that endogamous conjugations in this species (and he obtained many of them) are sterile because the micronucleus is lost through degeneration. I have preparations of this form made at intervals throughout the 665 generations, during endogamous and exogamous conjugation, at periods of depression and of vigor, and in none of them is the micronucleus absent. Upon this point Maupas was most assuredly mistaken.

From time to time As and Bs have been put together for the purpose of obtaining fertile conjugations. In the material set aside as "stock" conjugations have also frequently occurred, these of course, being endogamous. A comparison of "wild"

[1] Cf. Calkins. Studies on the Life-Cycle of Protozoa. I., The Life-Cycle of *Paramœcium caudatum*. *Arch. f. Entwickl.*, 1902.

conjugations with endogamous and exogamous unions is very instructive. By "wild" is meant pairs that are captured while conjugating in the natural pond water. There were only three pairs of these but of the six ex-conjugants five continued to live in the regular culture medium. Had the original number of pairs been larger, the result would be more satisfactory but I believe the proportion of fertile results would be as high as five out of six, or, expressed in percentages, as high as 83 per cent. Of the exogamous conjugations, *i.e.*, those of diverse ancestry, there were 24 pairs selected at different periods, and 48 ex-conjugants isolated, of which only three continued to live in the same culture medium, or about six per cent. Of the endogamous unions, *i.e.*, conjugations in which the gametes were of the same ancestry, 16 pairs in all were selected and 32 ex-conjugants isolated, of which two only continued to live, again about six per cent. How are we to account for the great discrepancy between the proportion of fertile wild ex-conjugants and those obtained from the cultures? *I believe that it can be accounted for on the ground of chemical differences in the composition of the protoplasm of the different individuals.* In the natural habitat of these forms no two individuals have the same environment for long periods and it may well be that no two parts of the pond have an identical composition. When two organisms unite, as *Paramœcium* unites, and part of the micronucleus of one unites with a similarly-derived part of the micronucleus of the other, the result is the formation of a new chemical substance in each organism, for the fusion nucleus in all probability is different in minute composition from either of the old ones. Now, on the other hand, when the organisms had been in the same culture medium for long periods, as was the case with the As and Bs of these experiments, there was no chance for them to be different from one another in chemical composition, and the ex-conjugants, after a few generations, died in the great majority of cases. In all such cases the conjugations were normal, micronuclei were exchanged, the old macronuclei became disintegrated and new ones were properly formed, but something was lacking and the ex-conjugants died. I believe this "something" has to do with the chemical make-up of nucleus and cytoplasm; and I further believe that the failure

which Maupas experienced in getting fertile endogamous ex-conjugants can be explained upon the same grounds. In one instance I treated an endogamous ex-conjugant with beef extract. The effect was most remarkable for it not only continued to live, *but it is still living in the 350th generation.* When it is remembered that the usual life-cycle of *Paramœcium* in culture is from 150 to 170 generations, this result is extremely interesting.

The facts of conjugation thus seem to fit in with the interpretation of the other results, to indicate that the protoplasm of this one-celled organism, like a galvanic battery, starts with a high potential of activity, which gradually fails with use, but which may be restored again and again by the proper chemical means. It is to such an assumption that we may turn for an explanation of the periods of depression that have occurred from time to time. The June period, at the 620th generation, appears to have been different from the earlier ones, for at this time, neither beef-extract, nor any of the salts that had been successful before, would restore the lagging energies. It was only the use of something entirely different from everything previously employed that finally saved the culture. It may have been the lecithine and allied substances in the brain extract, and it may have been the nucleinic acid in the pancreas extract; whatever it was, it apparently supplied something in the protoplasm of *Paramœcium* that had not given out before, but had failed at this particular time. Further, and more carefully-guarded experiments, must now be undertaken in the hope of finding out what these failures mean.

Turning again to the controversy regarding "old age" in Protozoa, it seems that we shall have to side with Weismann in the contention that these organisms have the potential of endless existence, and this without conjugation. The five times that the cultures of *Paramœcium* have been rescued at periods of depression, justify the belief that they may be saved as many times more, provided the time and patience required for such work are not out of all proportion to the results gained. It seems to be merely a matter of finding the right food, *i. e.*, the right chemicals. In nature, it is not improbable that the periods of depression, which are experienced in the laboratory, would be

absent, and that the division-rate would maintain a fairly uniform level. The chemical composition of a pond is constantly changing; every rainfall washes new salts into the water; every wind assists in turning the water over, and with these changes, it is probable that the organisms get new supplies of the substances necessary for their continued activities. When other things fail, the organisms may resort to conjugation, but we may consistently doubt whether this method of recuperation for Protozoa is as widespread in nature as hitherto supposed.

One other analogy is suggested by these experiments, *viz.*, the similarity of these periods of depression, and the subsequent proliferation, to tumorous growths of various kinds in the higher animals. The race of *Paramœcium* may be compared with the tissues composing the body of a Metazoön. When the cells composing these tissues approach the age-limit of the race, they divide less and less often; finally they come to a halt, so far as division is concerned, and senile degeneration begins, which ends in death. If, in a given tissue, after it has passed the period of active division, some stimulant be given at a local area, thus inducing a renewal of the dividing energy and proliferation of the cells of that area, the result, from a pathological point of view, would be a tumor; from a biological point of view, it might well be the rejuvenescence of the cells, as in the case of *Paramœcium*. As in the latter case, a number of agents might be responsible for calling out the increased activity, as, for example, a blow, a parasite, an enzyme, a changed condition of the immediate environment, etc. Any one of these might "rejuvenate" the tissue-cells in the same way that a chemical induces a new cycle of generations of *Paramœcium*.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLL, August 6, 1902.

FURTHER NOTES ON THE COCOONS OF ALLOLOBOPHORA FŒTIDA.

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The fact that cocoons of *Allolobophora* are formed during copulation challenges the interpretation that spermatozoa are stored in the spermathecæ in order to produce cross fertilization. We found, however, such conclusive evidence that the spermatozoa of the cocoon are derived from the spermathecæ, that this apparent inconsistency was stated in an earlier paper as a problem for further investigation.[1]

During the summer of 1901, we were able to make at least one step in this direction. We found that cocoons can be formed and deposited when the worms are not copulating. Isolated worms deposited cocoons for weeks, as many as ten cocoons being laid by one worm without copulation.

The method of experimenting was as follows: Single worms with a pronounced clitellum were isolated in half-pint bowls, each bowl being half filled with compost in which the worms had been found. Immediately after this compost was collected, it was carefully sifted to get rid of even the smallest worms, and it was then kept for three or more days before using. This was done to allow any cocoon already in the compost to reach a stage of development that would make it impossible to mistake it for a cocoon laid by the worm isolated for the experiment. At the end of three days the isolated worm was put in a bowl of fresh compost and the compost in which the worm had been kept was sifted under running water through a sieve with meshes too fine to allow a cocoon to pass. By this process nearly all the earth was washed away, and any cocoons present, were easily secured.

A perfectly fresh cocoon, *i. e.*, one with a slime-tube, was often found, but more often the cocoons contained eggs in a later

[1] The Cocoons and Eggs of *Allolobophora fœtida*, Jour. Morph., Vol. XIV., No. 3, 1898, pp. 496, 497.

stage of development, 2, 3, 4, and even 10 or 12 cells. When the stage of any egg appeared doubtful, it was fixed, stained and mounted for examination. Any cocoons in the compost before it was put in the bowls would have had a start in development of at least three days — so it was practically impossible to mistake them for the cocoons laid by the isolated worm.[1]

Twenty worms were isolated for the experiments described, and of these, one worm deposited ten cocoons at average intervals of three days. This worm was vivisected six days after depositing the tenth cocoon, and all four spermathecæ were found to be about one quarter full. The clitellum — which was very pronounced when the worm was isolated — had entirely disappeared, and the seminal vesicles appeared abnormal — they were a brownish-yellow, and quite different in appearance and texture from those of worms captured in the compost heap. This pathological condition, undoubtedly due to confinement, may have prevented the deposition of cocoons continuing until the contents of the spemathecæ was exhausted.

Of the remaining nineteen worms, one deposited four cocoons, and one deposited three, within fifteen days ; one worm deposited three in nineteen days ; two deposited two within seven days, and two deposited two within eight days. Three worms deposited each one cocoon on the third day, one worm deposited one on the fourth day, and one deposited one on the fifth day. In some of these cases the experiment was cut short by the escape of the worm.

Seven of the nineteen worms deposited no cocoon, vivisection showing, however, that two of them were pathological, and that the spermathecæ of four of them were almost empty. We found the spermathecæ full, and the seminal vesicles normal in only one of these worms, and there was no apparent cause why this worm should not deposit cocoons. These worms were isolated from thirteen to twenty days before vivisection.

The experiments described above establish the fact that a

[1] In order to become perfectly familiar with the different stages of development possible within three days, we preserved a large number of fresh cocoons, opening them at intervals of three hours, and fixing, staining and mounting the eggs.

cocoon can be deposited by one worm alone, and our earlier observation of the formation of cocoons during copulation, seems to prove conclusively that there are two methods of forming cocoons. These observations reconcile the opposing opinions[1] on this point.

That the deposition of cocoons by a single worm was not due to isolation, we determined by finding, in pots containing one

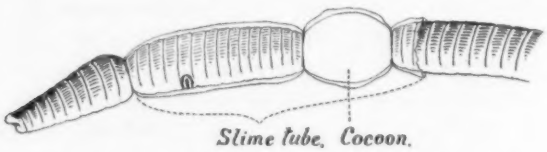

FIG. 1. × 3. Forty-two anterior segments of a worm with a cocoon in the process of formation. The transparent slime-tube surrounds the worm from the tenth to the thirty-sixth segment, and shows constrictions at each edge of the clitellum. The white cocoon encircles the clitellum. When the worm was dropped into boiling water, the cocoon became opaque — effacing the segments of the clitellum.

hundred worms, a number of single worms each with a cocoon around the clitellum. During the summers of 1901 and 1902, we found sixteen of these worms, with cocoons in different stages of development, ranging from a cocoon containing only a trace of albumen and no eggs to one with the full amount of albumen of a mature cocoon, with 28 eggs. These immature cocoons, when removed from the worm, are surrounded by a single slime-tube, resembling one half the double tube found around a copulating pair. This single cocoon lies near the posterior end of the slime-tube (Fig. 3), maintaining the relation that exists between the slime-tube and cocoons in the case of copulating worms, where the two cocoons lie at opposite ends of the double tube. Examination with a lens shows the clitellum of the worm encased by the cocoon and part of the slime-tube, the longer part of the tube lying anterior to the clitellum (Fig. 1). When the worm is not disturbed it will withdraw backward, through the slime-tube and cocoon, finally pulling the head through the smaller end of the cocoon (Figs. 2 and 3). This accords with

[1] We have not been able to find any record other than ours of actual observation of the deposition of cocoons by copulating pairs, or of single worms with cocoons in process of formation.

our observation of copulating pairs, for in these cases also, the longer part of the slime-tube lies anterior to the clitellum, each worm finally withdrawing backward through the small end of the cocoons. In the worm shown in Fig. 1, the slime-tube extends to the tenth segment, but it is not safe to assume that in the living worm the tube may not extend still further, possibly covering the spermathecal openings between the ninth and tenth, and tenth and eleventh segments. The sudden

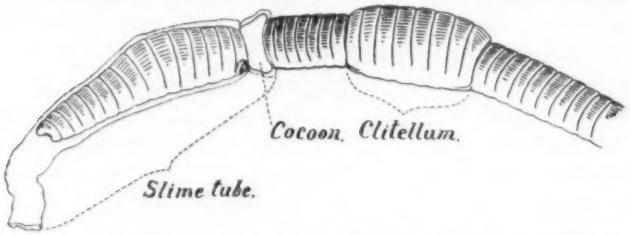

FIG. 2. × 3. Forty-two anterior segments of a worm caught with a nearly mature cocoon around its clitellum. Before being dropped into boiling water, the worm partly withdrew from the cocoon, leaving it encircling the 16th–18th segments. The cocoon and segments are very much contracted, the cocoon appearing as a narrow, opaque band around the worm. Such cocoons regain the typical oblong shape when removed from the worm. The worm has partly withdrawn from the slime-tube, leaving the anterior end projecting beyond its head.

extension of the worm, when dropped into boiling water, possibly causes such change in the position of the slime-tube, as shown in Fig. 1.

On the other hand, we have no proof that the contractions of the worm when captured do not result in an extension of the tube beyond the point covered under normal conditions, and that in the living worm the tube does not reach the spermathecal segments. In living worms, we have seen the slime-tube, in some cases, stop short of these segments, in others, completely cover them, but it is impossible to affirm which condition prevails when the worm is at rest. Fig. 3 shows a tube with its posterior limit clearly indicated, by the impress of five segments posterior to the clitellum. A comparison with Fig. 1 shows that the slime-tube in the captured worm stops short of this point.

Our method of securing cocoons in the process of formation

on single worms, was to dump daily several pots, each containing one hundred worms and carefully to examine the clitellum of each worm. The presence of a slime-tube, with or without a cocoon, can be detected, by constrictions at each end of the clitellum. These constrictions are very marked when the worm is contracted and difficult to see when the worm stretches out. When the worm succeeds in partially escaping from the slime-tube, one or both constrictions can be seen on, or near, the clitellum. These constrictions are produced by the slime-tube and

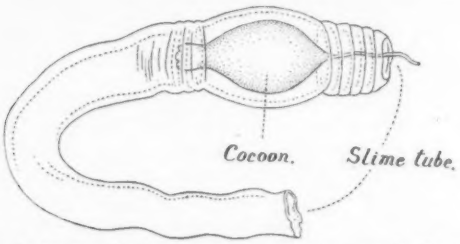

FIG. 3. A freshly deposited cocoon. When on the worm the shorter end of the slime-tube is posterior to the clitellum and the impress of 5 segments on the tube shows that, in this case, the posterior end of the slime-tube extented to the 39th segment of the worm. This figure was reduced one fourth from Fig. 3 of an earlier paper. The original figure was magnified seven diameters.

not by the cocoon, for we have found worms showing marked constrictions, when careful examination of the slime-tube after removal, failed to show any trace of a cocoon. This indicates that the formation of the slime-tube is the first step in the process of forming a cocoon.

Having established the fact that cocoons are formed by these worms, during copulation or separately, the opposing opinions on this point are harmonized. The question now remains, which method predominates?

We find eggs in the cocoon while it still encircles the clitellum, proving that they must be conveyed through the slime-tube, from the oviducts on the 14th segment, to the cocoon; and that they are not deposited in the cocoon when it finally passes over these segments, as suggested by several investigators.

We have found from one to twenty-eight eggs generally near the anterior end of the cocoon. In some cases these cocoons

were so little advanced in development that without a lens they could not be detected until the slime-tube was removed from the worm. Examination of the tube revealed an immature cocoon, with a white semi-transparent covering, containing a much smaller quantity of albumen than is found in mature cocoons, but the eggs showed the stage of development found in a freshly deposited cocoon, *i. e.*, the first maturation spindle at the metaphase and in the center of the egg. Evidently the delicate covering of the cocoon is formed before the albumen is deposited, but the substance that forms the chiton of the covering of the older cocoons is not present, for we have preserved these immature coverings in moist earth for twenty-four hours, and they failed to become yellow and tough, like the covering of the mature cocoon under these conditions; they were white and soft at the end of the experiment.[1] When the slime-tube is torn from these immature cocoons, we find the covering of the cocoon consists of two layers; an outer, perfectly transparent layer, that can be slipped off the inner covering, which is thinner, more resistant, and semi-transparent. The former appears to be part of the slime-tube substance that adheres to the cocoon, but in later stages, when the cocoon is ready for deposition, this layer if preserved becomes yellow like the under layer, which indicates it to be part of the covering of the mature cocoon.

Spermathecæ.

In 10 per cent. of a large number of worms dissected, each with a pronounced clitellum, all four spermathecæ were found empty, this proving that the appearance of the clitellum does not indicate whether the spermathecæ are full or empty, and also that cocoons can be formed until the spermathecæ are entirely empty. In a few cases we found the spermathecæ of copulating worms empty, even when several spermatophores were present in the slime-tube over the spermathecal segments, showing that, spermatozoa are not received into the spermathecæ during the early stages of copulation.

The condition of the individual spermathecæ varies greatly. Sometimes we found only one of the four entirely empty, and

[1] In only one case, an immature cocoon, with a trace of albumen and one egg, showed this characteristic yellow tinge after three hours in water.

again only one full, while the contents of the other three varied in amount. We were unable to discover any constancy in these relations.

Spermatophores.

Having often observed the deposition of cocoons by copulating worms, one of us[1] was led to conclude that the spermatophores in the double slime-tube represented the first step in the formation of the two cocoons. If this were true, we should find spermatozoa in immature cocoons at the earliest stages. The albumen from the immature cocoons found around the clitellum of single worms was dried on slides, stained with iron hæmatoxylin and examined under a Zeiss 4-mm. and a 2-mm. immersion lens, with the aid of a mechanical stage. This albumen resembled that found in freshly deposited cocoons, but *did not contain* spermatozoa. It is evident from the above, that the spermatozoa are put into the cocoon at a later period, possibly when the cocoon passes over the spermathecal openings as it is deposited. The fact, however, that the slime-tube shown in Fig. 1 extends to the posterior spermathecal openings suggests that in its normal position the tube *may* extend to enclose all four spermathecal openings, and the spermatozoa like the eggs be conveyed through the tube to the cocoon, before deposition. But the albumen of three cocoons apparently just ready for deposition failed to show any spermatozoa. These cocoons tightly encircled the clitellum and were equal in size to a freshly deposited cocoon; one contained eleven eggs, two normal; one contained twelve eggs, six normal; and one contained twenty-eight eggs, five normal. The white covering of one cocoon after one hour; of one after twenty minutes; and of one after ten minutes, in water, became tough and yellow like the fresh cocoon a few minutes after deposition.

That spermatozoa are not found in nearly mature cocoons, and that spermatophores *are* found in the slime-tube of copulating worms before the covering of a cocoon is formed, proves beyond question that the spermatophore is not a device for conveying spermatozoa into the cocoon. It would seem rather to be a device for filling the spermathecæ, and it is not formed *after* the spermatozoa leave the spermathecæ, as conjectured in an earlier paper.[2]

[1] *Loc. cit.*

[2] *Loc. cit.*, p. 498.

Spermatophores, however, are certainly not received into the spermathecæ in the form of spermatophores, for we have vivisected many copulating worms having spermatophores at each end of the slime-tube (*i. e.*, over the spermathecal openings of both worms) and the contents of the spermathecæ, in every case, was quite unlike the spermatophore substance; the former mixing freely with water, while the spermatophores do not disintegrate even after twenty-four hours in water.

The granular substance secreted by the spermathecal glands[1] which forms the center of the spermatophores also fails to disintegrate in water, and we have not been able to identify this substance in the spermathecæ.

When copulating worms are allowed to withdraw from the double slime-tube, we sometimes find aggregations of spermatophores in the definite areas of the slime-tube where the two cocoons are formed. The constrictions of the slime-tube that close each end of the cocoons, confine the spermatophores to these areas, and when filled with spermatophores, these areas bear such a striking resemblance in form and color to freshly deposited cocoons, that in our earlier observations we misinterpreted them as partly formed, or nearly completed cocoons. The true "partly formed cocoon" has a white semi-transparent covering, contains more or less albumen and several eggs. Prior to 1901 we had found these cocoons only in the compost not around the worm. We found only a few, and in every case the cocoon contained eggs, but as we did not preserve the albumen, the absence of spermatozoa was not discovered and we supposed these cocoons to represent a later stage of development than the aggregations of spermatophores in the slime-tube.

The marked difference in structure of the spermatophore substance and the contents of these cocoons was noted, as well as the fact that in freshly deposited cocoons we found only a very small number of spermatozoa, in comparison with the large quantity found in a slime tube containing spermatophores. It was these inconsistencies that prompted us to undertake the experiments described above.

[1] *Ibid.*, p. 496.

A BIOLOGICAL FARM.[1]

For the Experimental Investigation of Heredity, Variation and Evolution and for the Study of Life-Histories, Habits, Instincts and Intelligence.

C. O. WHITMAN.

The biological laboratories of to-day, in design, equipment and staff, are almost exclusively limited to the study of *dead* material. Living organisms may find a place in small aquaria or vivaria, but they are reserved, as a rule, not for study, but for fresh supplies of dead material. It is no disparagement of the laboratory to point out a broad limitation in its ordinary functions and the pressing need of new facilities for observation and experiment on *living* organisms.

The fundamental problems of heredity, variation, adaptation and evolution cannot be wholly settled in the laboratory. They concern vital processes known only in living organisms—processes which are slow and cumulative in effects, expressing themselves in development, growth, life-histories, species, habits, instincts, intelligence. These problems require, therefore, to be taken to the field, the pond, the sea, the island, where the forms selected for study can be kept under natural conditions, and where the work can be continued from year to year without interruption. Such a field, combining land and water, and stocked with animals and plants, and provided with a staff of naturalists, would have the essentials of a biological farm, now justly considered to be one of the great desiderata of biology.

This great need (pointed out in all our annual programmes since 1892, and named as one of the three leading purposes of the Culver endowment) has been felt ever since Darwin's time, and has been strongly urged by such evolutionists as Romanes, Varigny, Galton, Weismann and Meldola. Thus far the project has not been realized, except on a small scale through individual effort.

[1] Read to the Corporation of the Marine Biological Laboratory, at the annual meeting, August 12, 1902.

The most notable move in this direction is that of Professor Cossar Ewart, of the University of Edinburgh. "The Penycuik Experiments"—the first product of Professor Ewart's enterprise—form a brilliant illustration of the kind of fruit to be expected from a farm devoted to experimental research. Single-handed, Professor Ewart attacks the problems of heredity, and quickly shows how decisive are direct experiments in dealing with such subjects as telegony, prepotency, reversion, inbreeding, etc.

The plans proposed by Romanes and Varigny had as chief ends in view demonstrative tests of the theory of the origin of species by natural selection. But the contest between the old belief in the immutability of species and the new doctrine of descent has been decided, and the original idea of the farm has consequently ceased to have great influence.

The functions to be fulfilled by a farm are no longer prescribed by the exigencies of theories, but by the deeper and broader needs of pure research on *living* organisms. The problems of heredity and variability are fundamental, and naturally form the center of interest. Variability is the source of new species and the fountain of all progressive development in the organic world. In heredity lies the power of propagation and continuity of species. These are inexhaustible subjects, from the investigation of which must flow rich accessions to knowledge, which will redound to the advancement of human welfare.

These subjects are in some aspects and details amenable to laboratory research; but for the most part they can only be effectively dealt with under conditions represented in the farm. This holds, for example, in that most promising branch of experimental biology—*hybridisation*. Botanical gardens and zoölogical parks have been utilized to some extent in this work, but they are adapted to show-purposes, and of little value for research of this kind. The far-reaching importance of this subject, both for science and practical breeding purposes, is well attested in Mr. Ewart's experiments, in those of Hugo de Vries, as recorded in his monographs on the origin of species in the plant world, and again in Mr. Bateson's "Experimental Studies in the Physiology of Heredity."

The functions of a biological farm are not all summed up in experimentation. That old and true method of natural history—*observation*—must ever have a large share in the study of living things. Observation, experiment and reflection are three in one. Together they are omnipotent; disjoined they become impotent fetiches. The biology of to-day, as we are beginning to realize, has not too much laboratory, but too little of living nature. The farm will certainly do much to mend this great deficiency. The farm would enable us to work out life-histories, bring us face to face with instinct, put it under control, so that we could handle it, photograph it, analyze it, read its history, and extort from it an answer to the question, Whence and how came intelligence?

It would enable us to extend the study of development beyond the stages represented in the egg and the embryo to those leading up to mature age, and thus bring within reach vast series of most important data for the study of evolution.

In such data we might expect to see to what extent individual development recapitulates race development, and to get important clues to the meaning of this so-called biogenetic law. The whole meaning of development and heredity is involved in these phenomena of "recapitulation." That the first step in recapitulation is the germ-cell, we know. The fertilized germ, or egg, passes through a series of form-stages, leading through the morula, blastula, gastrula, embryo, larva, etc. Whether these stages epitomize the ancestral series is a question very difficult to decide, and opinion is much divided. This obscure but fundamental problem of development can probably never be solved by embryological data alone. Paleontology throws much light on the general question, but deals entirely with non-living remains. If there be recapitulation, it should certainly be discoverable in post-embryonic stages, where characteristic features are slowly elaborated and brought to a completion in detail quite beyond the possibilities in earlier life. Strange to say, these later stages have been but little studied in living forms, museum morgues having been the chief reliance. It is in these stages that recapitulation may be actually seen as a life-process, successive steps in evolution repeating themselves with sufficient fullness to satisfy the most skeptical. Such sequences are often manifest in the

development of instinctive behavior, and even in voice-changes and food-instincts at certain life-epochs corresponding seemingly to evolution-epochs. Remembering that the distant ancestors of land animals were undoubtedly aquatic, the history of individual development in amphibious forms of to-day becomes intelligible as an abbreviated and variously modified record of race development. Making all allowance for secondary adaptive changes, it is nevertheless safe to say that race evolution is sketched in the development of the individual—sketched not only in fundamental features of structure, but also in the accompanying physiological and psychological changes.

Reminiscences of aquatic life are seen not only in land animals that return to the water to deposit their eggs, but also in all the higher animals, since they begin life in the unicellular stage and require for their first development to be bathed in fluid.

Sequence in color-patterns, so characteristic of young animals of almost all species, and especially so of birds, furnishes innumerable illustrations of the biogenetic law, and in many cases, where only two extremes of the sequence are present, it is possible by simple experiment to bridge the gap, and thus to show that the two extremes are really two stages of a continuous development. For example, in some wild species of pigeons we find that the color-pattern of the first plumage succeeding the down is so different from that of the second (adult) plumage, as to appear to have no direct developmental relation to it. By plucking one or more feathers from the first plumage at differen times before the first moult, intermediate stages can be obtained, showing precisely how the first pattern can be progressively converted into the second. Such experiments enable us to force from nature more complete records of her past and present doings.

Work on living organisms, dealing with such subjects as heredity, variation, adaptation, correlation, development, recapitulation, hybridisation, origin of species, nature of specific characters, life-histories, habits, instincts, intelligence, etc., requiring uninterrupted continuance from year to year for long periods, under conditions that secure most favorable control for experimentation and study, calls for facilities which have yet to be provided.

There is no quite satisfactory name for the new plant required for such work, and no one has suggested a practical method of developing it. Biological Farm, broadly defined, is perhaps the best we can do for a name, as the work would be, so far as possible, upon plants and animals under cultivation. A considerable tract of land, of varied surface, including woods, fresh-water ponds, some brackish water, and a stretch of sea-shore that could be utilized in the cultivation and study of marine animals, would represent the essentials of the farm headquarters.

The best location for the farm would be in the immediate vicinity of a laboratory holding the position of a national center of biological research. The Marine Biological Laboratory has a good prospect of becoming such a center, if it is not one already, and its proximity to the United States Fish Commission Station only insures additional advantages, of great importance to the farm. The farm, the laboratory, and the station would be reciprocally helpful and stimulating, and in many problems the three could work hand in hand, each supplementing the work of the others. These three establishments would form a most comprehensive biological center, such as has never been equalled.

In dealing with the problems of heredity and variation, it is of the highest importance to know the *history of the material* to be investigated. It is this prime essential that is so conspicuously missing in most of the work hitherto done in these lines. Curves and formulæ may be all right mathematically and yet all wrong biologically. Even Galton, the father of the statistical school, warns us that "no pursuit runs between so many pitfalls and unseen traps as that of statistics." (Biometrika, I., p. 8.)

The farm will furnish material with *exact records*, and will thus render a most important service to laboratory workers. A single illustration will suffice. It has been discovered that the paternal and maternal chromosomes in the cross-fertilized egg remain distinct, at least in the earlier stages of development. This seems to account for the fact that hybrids of the first generation between distinct species are generally "intermediates." When these hybrids breed *inter se* or with the parent species, we often get so-called "reversions." Hitherto we have not found any explanation for these "reversions." The solution of this

most interesting problem in heredity only waits for the right material with precisely defined origin, and for this the laboratory could look to the farm. But the farm would do more than supply the needed material; its records and experiments would suggest the theory and give the physiological test, while the laboratory work would find the morphological test. The coöperation between laboratory and farm would thus be intimate and of inestimable value in a multitude of ways.

It must be remembered, moreover, that Wood's Holl has great natural advantages as a location for the farm. Ever since Baird's time it has been generally known that this is the best place on the Atlantic coast for the study of marine biology. Indeed, Wood's Holl has become a strong biological center by virtue of the exceptional opportunities for work here offered in a varied and extensive shore fauna and flora, in numerous accessible islands rich in forms of peculiar interest, and in many perfectly isolated fresh-water ponds, brackish ponds, and salt-water ponds of easy control. These attractive features, together with a climate suited to both winter and summer work, certainly comprise the essentials of a good location.

A further consideration in favor of this location is the fact that here for the first time in this country the farm project was definitely formulated; and, what is more to the point, it is here that the first step in the development of a farm has been taken, and the work carried forward for some six years by individual endeavor. The birthplace of an enterprise is not likely to be a pure accident. In the present case it was certainly determined by the various causes which have conspired to make Wood's Holl a biological center. This center has at least fifteen years of growth, and every year makes it stronger and increases its importance as a location for the headquarters of a biological farm.

The argument for location has already suggested that the farm is not necessarily limited to a single tract of land. It is designed to supplement, and coöperate with, a laboratory, and hence must have its headquarters conveniently near. There is no reason, however, for limiting its territory to the ground selected for this purpose. If, to take a familiar example, a study of the terns were to be undertaken, we should undoubtedly resort to Peni-

kese, taking advantage of the headquarters selected by the birds. If we were to take up some groups of migratory birds, we might find it desirable to migrate with them, changing our location to suit their summer and winter predilections. For forms settled in tropical regions, longer excursions might be necessary, and this might lead to the development of a new farm. These possibilities do not in the least conflict with the plan proposed for Wood's Holl. No matter where a farm be located, it must have headquarters, and occasionally extend its field of work to more or less distant points of interest.

The headquarters must be in close touch with a laboratory, and both should be in a place where the natural advantages and the organization are such as to draw a large number of investigators — most emphatically not in a place that invites a large number of spectators, as in the public parks of cities. In this latter respect Wood's Holl is most perfectly adapted to the purpose, and the prospect is good that we shall never be troubled with throngs of summer visitors. This is an ideal feature in the situation that it would be hard to duplicate.

The practical question arises as to how to proceed with the development of a farm. Our limited experience strongly confirms the opinion with which we set out, namely, that the best method is to develop the farm slowly, section by section. Each section should be a group of related species, selected with a view to combining a wide range of problems. It should be developed and directed by an investigator prepared to make it his life-work. This investigator, or director, should have the support of a number of assistants competent to deal with special problems, one or two artists, a photographer, a stenographer, a keeper and a business superintendent.

Developed in this way, the cost of maintenance would not be heavy at first. Ten thousand dollars a year would support a large and thriving section. The multiplication of sections and the gradual growth of the work would call for a larger income. A farm of ten large sections would require an endowment of a million, and it is easy to see room for many millions.

If the scheme here outlined approaches the ideal which science is waiting to see realized, it will be seen that the farm does not

find its chief purpose in demonstrations of the truth of evolution or in testing the theory of natural selection. It is not a project designed simply to turn out curves and formulæ for the delectation of the knight-errants of statistical lore, nor is it the particular pet of any school or fad. Moreover, the prevailing idea that it has something in common with a zoölogical or botanical park rests on a total misapprehension. The organization, management and all the conditions obtaining in the public park are incongruous with those required for a research farm. Heterogeneous collections of animals, exhibited for the amusement of people, are wholly unsuited to the purposes of investigation in time, place and character. For the kind of work contemplated, the investigator must have forms of his own selection, collected, arranged and kept for his special purposes. He must have complete and permanent control of his quarters and the forms he is to study, and above all, *complete isolation from the public*. Only under such conditions could he have the unbroken quiet required in delicate observation, or expect natural behavior from the forms occupying his attention.

The farm project, let me say in conclusion, is one we cannot afford to see drawn away from Wood's Holl. It is an undertaking born and nurtured here, on a small scale to be sure, but still sufficient to give results and make clear the direct path to a large and most important development.

Hitherto we have felt perfectly secure in our right and ability to control the direction and scope of our development. Whatever compensation we may receive for the surrender of rights that go with the transfer of ownership to another institution, let us have no illusions. We shall henceforth only be a few among many advisors on the questions that concern the scope of our work and the enterprises that shall be permitted free development here. We shall undoubtedly be left quite free to manage the details of our work, but the decision of matters of far more fundamental concern to all who are deeply interested in the ideal development of a real *biological* center here — questions of the broader limits and character of our work — the decision of all this will certainly not be wholly with us. Not to realize this, would be woful blindness to the situation. The extent to which we can have and

hold our ideals will depend on the influence we can bring to bear, and in this all-important matter the Corporation can have, and will have, I trust, earnest work to perform.

Summary Statement.

Laboratories.—The biological laboratories of to-day are almost exclusively devoted to the study of "pickled" animals and plants. They have very few and inadequate facilities for the study of *living* organisms. This is a limitation for which no remedy has thus far been provided.

Field-work.—For the study of development, growth, life-histories, species, habits, instincts, intelligence, heredity, variation, adaptation, hybridisation, etc., we can neither depend upon the laboratory nor upon the field-work of naturalists. A world-wide field in which there can be no *control* of the forms to be studied, and no possibility of *continuity* in observation or opportunity for experiment obviously does not meet the requirements of science.

Biological Farm.—The laboratory is too narrow, and the world too wide for the continuous study of living organisms, under conditions that can be definitely known and controlled. For such study, selected groups of organisms and a limited territory, with favorable conditions of land, water and food are needed. Territory, living organisms and scientific staff would constitute a new plant, which might be called a *Biological Farm.*

Grounds.—The grounds of a biological farm would vary in extent with the growth of the work, from ten to a hundred or more acres. Land, woods, fresh-water ponds, sea-shore and islands would make a good combination.

Location.—The location of headquarters should be at a biological center, near laboratories drawing a large number of investigators, but at a safe distance from any large city or summer resort. These prime advantages of situation superadded to the exceptional natural advantages of available grounds are represented at Wood's Holl.

Natural Conditions.—A biological farm should include the largest possible diversity of natural conditions to insure wide freedom of development and opportunity for experimentation.

Water is the first essential, and three forms of this element are needed, namely, sea water, brackish water, and fresh water. All three forms abound at Wood's Holl and vicinity. The fresh-water ponds are numerous, and many of them, both on the main-land and on the neighboring islands, are *completely isolated* and stocked with forms in-bred for centuries. Brackish water in almost every degree, pure sea-water and tide-currents are right at hand.

Land is the next essential, and here we have hill, plain, marsh, swamp, shore and islands, and some of these islands are inimitable biological farms of nature's own make.

A seasonal range of temperature is essential to the existence of a majority of the forms best suited to cultivation and study; and in this are supplied very important conditions for experimental work. The surrounding sea protects Wood's Holl from the extremes of heat and cold.

Isolation.—The most favorable combination of conditions may be utterly worthless, unless the farm can be made secure in its isolation from the public. Its work must go on in a *quiet environment*, where all the conditions are under control, and the investigator is free from the danger of intrusion.

Mode of Development.—The work should be developed slowly, section by section, each section consisting of a group of related species, or a single species, offering a wide range of problems.

Each section should be in charge of a director, prepared to continue the work during life, and supported by assistants and help for all routine and mechanical service. The staff would consist of directors, assistant investigators, artists, photographer, clerical help, keepers and a business manager.

Outlay and Maintenance.—The original outlay for land, stock, buildings, equipment, inclosures of land and water for isolation purposes, would vary according to the forms selected for study. From $50,000 to $100,000 would suffice for this. The maintenance of the first section, including salaries, accessions to stock, library, etc., may be estimated at $10,000 a year. The cost of additional sections would be about $5,000 each.

Ideal Center.—The association of three such institutions as the Marine Biological Laboratory, the U. S. Fish Commission Sta-

tion, and a Biological Farm would form an ideal biological center. Each would help and be helped by the other two.

Coöperation.—There should undoubtedly be several biological farms in the country. The larger universities might well have their own farms, and thus very extensive and effective coöperative work be carried on.

Use to Science.—The farm would enable us to approach all the fundamental problems of life from the two sides of observation and experiment on living organisms. It would furnish material for study with precise records, and make it possible to keep up continuity in the experimental study of heredity and variation.

Practical Utility.—The utility of such work is seen when we reflect on the practical results already realized in the multiplication and improvement of domestic species of animals and plants through cross-breeding, hybridisation and selection. We have very meager and uncertain knowledge of the laws of heredity and variation—laws which underlie all progress of the race.

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. X. THE MOVEMENTS AND REACTIONS OF PIECES OF CILIATE INFUSORIA.[1]

H. S. JENNINGS AND CLARA JAMIESON.

The present paper records an attempt to investigate by operative procedure the division of labor among the organs of locomotion of the ciliate body; to determine the part played by cilia of different position or structure in the usual movements and reactions. In many of the Ciliata the body is much differentiated, often bearing a number of different sorts of simple or compound cilia, variously distinguished as membranellæ, cirri, setæ, cilia proper, and the like. In such organisms as *Stylonychia* (Fig. 1) and *Stentor* these different structures have a characteristic distribution, and apparently differ markedly in function. Even in less differentiated infusoria a distinction between oral or peristomal cilia and body cilia can usually be made.

Observation of the behavior of these animals indicates that there is a division of labor among these cilia in the production of the usual movements. The organisms as they swim through the water follow a spiral course, usually swerving continually toward one side, but compensating the deviation thus caused by rotating on the long axis. The swerving is as a rule (though not invariably) toward the side away from the peristome or the oral cilia. The stroke of the oral cilia is of such a character as to tend to turn the body toward the side opposite to that on which they are situated, so that the swerving appears to be due to these cilia,—aided, as a rule, by the form of the body.

In reacting to most stimuli, these organisms turn toward a structurally defined side (see Jennings, 1900), and, as a rule, this is again the aboral side, or that opposite the oral or peristomal cilia. Observation indicates that this is due to the stroke of the oral cilia, or in some cases to a still smaller and more localized group of cilia. This has been set forth by a number of authors,—

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 56.

notably by Pearl (1900), in the case of *Colpidium*, and by Pütter (1900, p. 273), in *Stylonychia*. In *Colpidium*, according to the observations of Pearl, it is a group of cilia at the anterior end of the body which, invariably striking toward the oral side when the animal is stimulated by the electric current, cause the body to turn toward the aboral side. In *Stylonychia* it is the entire group of peristomal cilia that, striking toward the oral side, cause the body to turn toward the aboral side.[1] We can testify from our own observations that these relations seem clear. One naturally concludes, therefore, that the sidewise motion is determined by these peristomal or anterior cilia, and that if they were removed the motion would no longer occur. Massart (1901, p. 27), on the basis of Pearl's work, accepts the idea that the sidewise movement is caused by different cilia from the backward or forward motion,—aiding thus to distinguish between what Massart calls *phobism* (the backward movement in reaction) and *clinism* (the lateral movement).

The work here set forth was undertaken for the purpose of getting further light on this and related matters. If the anterior or oral cilia are removed by operation, will the organism no longer turn toward the aboral side when stimulated? And is the character of the spiral swimming modified by removal of such cilia? In general, does the removal of specific groups of cilia (with the accompanying alteration of the body form) have specific effects on the movements of the animal?

The behavior of pieces of infusoria has been studied by Verworn (1889) and Balbiani (1888).[2] The results of these investigators are of the greatest interest, but do not touch specifically the questions raised above. They found that pieces of ciliates move in general in the same manner as do the entire organisms. The reaction to stimuli by turning toward a certain structurally defined side, and the swerving toward a structurally defined side in the spiral course, in the uninjured animal, had not at that time been observed, so that these matters were, of course, not observed for the pieces. The questions proposed in this paper were therefore not touched by the experiments of Verworn and Balbiani.

[1] These are aided in the turning, according to Pütter, by the "running cilia."

[2] Balbiani's paper we have been unable to consult, so that we must depend upon the account of it given by Verworn.

In giving an account of the results of our experimentation, matters which were described by Verworn (1889) will not be dwelt upon, but the account will be confined to the points bearing on the questions raised above.

The operation of cutting the animals was done with a small knife, under the Braus-Drüner stereoscopic binocular, which has great advantages for such work. It was found best to cut the animals when resting against a clean glass surface. Most of the operations can be performed by the aid of some patience without great difficulty. In all cases the results set forth are drawn from experimentation and observation on a considerable number of specimens.

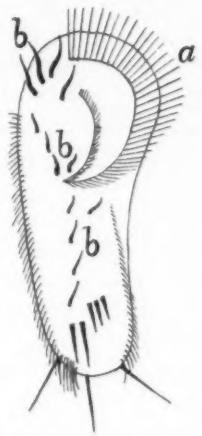

FIG. 1. *Stylonychia*, ventral surface, showing the distribution of locomotor organs (after Pütter, 1900).

1. *Stylonychia.*—Pütter (1900) gives a full account of the normal movements of this animal when uninjured, with a careful analysis of the action of the different cilia in producing the movements. For our purposes it is important to note the following different movements. (1) The animal runs forward on surfaces, chiefly or entirely by the aid of the "running cilia" *b*, Fig. 1. (2) When stimulated it jerks back a little, then turns to the right or aboral side. This turning appears to be due to the lateral stroke of the large peristomal cilia *a*. (3) The animal may also swim freely through the water; in doing so it swerves continually towards its right side, and revolves on its long axis from right over to left — the resulting path being a spiral. The swerving toward the right appears to be due to the lateral stroke of the peristomal cilia, and the revolution on the long axis might well be due to the same factor.

When *Stylonychia* was cut in two transversely just behind the mouth, so that the entire peristome remains with the anterior half, the pieces move as follows:

1. Anterior half, possessing peristome (Fig. 2, *a*).—After the first shock effects, the movement throughout is nearly like that

of the uninjured animal, but somewhat more rapid than usual. The piece runs forward on the bottom, turning to the right when stimulated. It differs from the entire animal in that it much more rarely jerks backward when stimulated, though it does this when the stimulus is powerful. When swimming through the water, it revolves to the right and swerves to the left, as in the entire animal. Since the anterior half possesses the peristome, the similarity of its movements to those of the uninjured animal was to be anticipated.

2. Posterior half, without peristome (Fig. 2, *b*).—Immediately after the operation the piece jerks rapidly backward, turning at the same time toward the right side. After a time it becomes quieter, and now it reacts essentially like the entire specimen, though it is somewhat less active. If stimulated with the glass hair at the posterior end its runs forward; stimulated at the anterior (cut) surface, it jerks backward and turns to the right. Chemical stimuli cause the same reactions. A little m/20 NaCl, or a weak solution of methylene blue was introduced with a capillary tube close to the piece; on coming in contact with the chemical the piece backs and turns to the right, just as is done by the entire animal.

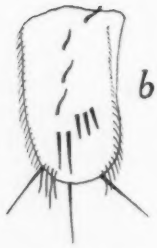

FIG. 2. Anterior (*a*) and posterior (*b*) halves of *Stylonychia*.

Thus the presence of the peristomal cilia is not the determining factor for the invariable turning to the right as a response to stimuli.

3. Posterior one fifth. — The movements are somewhat less well coördinated than in the larger pieces. But after the shock effects have ceased, the piece creeps forward, *turning to the right*. It swims slowly through the water, swerving to the right and revolving to the left, as in the uninjured specimen.

4. Anterior one third. — This is very rapid in its movements, and circles almost continuously to the right. In the free water it revolves in the usual way. When stimulated, it turns to the right, but *does not jerk back*.

5. Middle one half. — This was obtained by cutting off the anterior and posterior fourths of the body. Its movements and reactions are essentially similar to those of the uninjured specimen.

6. Right and left halves. — These were obtained by splitting *Stylonychia* lengthwise. They move and react (after the shock effects have ceased) in nearly the normal manner. Thus, when stimulated mechanically or chemically, they jerk backward, and the right half turns toward its right (uninjured) side, while the left half likewise turns toward its right (injured) side.

A specimen was cut lengthwise in such a way that the left piece was a strip comprising about one third the body. This moved somewhat irregularly, but reacted in the same manner as the left one half just described — though it evidently bore no part of the peristome.

7. A small, left posterior corner, comprising less than one fourth the animal, reacted, after the effects of the shock had ceased, in the usual way — by backing and turning toward the right.

Thus, on the whole, pieces of *Stylonychia* from any part of the body, if amounting in size to as much as one fourth to one half of the animal, move and react essentially like the entire specimen. In very small or very irregular pieces the movements become irregular, as might be expected.

Similar results were obtained with *Oxytricha fallax*, and with a number of unidentified *Hypotricha*, though the smaller size makes most other species less favorable for such experimentation than is *Stylonychia*.

II. *Stentor cæruleus*. — In *Stentor* the oral (or adoral) cilia or membranellæ form nearly a circle at the broad anterior end of the body. *Stentor*, when free swimming, follows a spiral path, revolving from right over to left. It reacts to chemical and mechanical stimuli by backing a little, then turning toward the right aboral side. It is evident to observation that the adoral cilia play a large part in the turning; from their large size and their posi-

tion at one end of the body it would be natural to conclude that the turning is due to them alone.

1. The entire anterior disk, with the adoral cilia and the mouth, was removed by a transverse cut near the anterior end (Fig. 3). The posterior portion (*b*, Fig. 3), having only the body cilia, swam through the water as usual, in a spiral, revolving from right over to left. The aboral side in *Stentor cæruleus* is usually curved in a very different manner from the oral side, so that it is possible to distinguish the two sides even when the disk is removed. The specimen deprived of the disk and anterior cilia reacted to mechanical and chemical stimuli by backing and turning toward the aboral side, just as does the uninjured specimen.

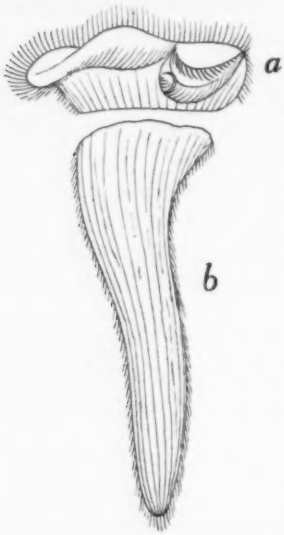

FIG. 3. *Stentor*, cut transversely just behind the disk.

After a time the specimen attached itself by the foot, and extended in the usual manner. Currents, due to the ciliary action, then passed backward toward the foot, or at times they were reversed, passing in the opposite direction. There was, however, no evident whirlpool formed, with a definite center, as in the uninjured specimen. The animal from which the disk had been removed, after becoming attached, reacted to mechanical stimuli by turning into a new position, and later by contracting, just as happens in the uninjured specimen. (On the behavior of *Stentor*, see Jennings, 1902.)

2. The disk portion (Fig. 3, *a*), bearing the adoral cilia and the mouth, also moved and reacted in the usual manner—revolving to the left as it swam, and responding to stimuli by backing and turning toward the aboral side.

3. Posterior one fourth—comprising about one half the slender "stalk" of *Stentor*.—This swims about, revolving to the

left. Whether on stimulation it turns toward a definite side could not be determined. After a time the small piece fixed itself by the foot and extended. The currents caused by the cilia were most of the time directed away from the attached end, being thus the reverse of the normal currents. At times, however, the current passed toward the foot.

When stimulated mechanically, the piece responded, like the entire *Stentor*, by bending into a new positiom, and later by contracting.

III. *Spirostomum ambiguum.* — Essentially the same results were obtained with this large infusorian as with *Stylonychia* and *Stentor*. Both the anterior half, bearing the adoral cilia, and the posterior half, without adoral cilia, move and react in much the same way as does the uninjured individual. They revolve to the left when swimming freely through the water, and turn toward the aboral side when stimulated. The anterior half moves more rapidly than does the posterior half, and when stimulated turns through a much greater angle than does the latter. The posterior half, if not strongly stimulated, frequently remains nearly quiet, or oscillates back and forth, as described in the third of these studies (Jennings, 1899). If stimulated mechanically or chemically, however, it backs and turns toward the aboral side.

A posterior one third of the body reacts in the same manner as the posterior one half.

A minute piece from the posterior end, less than half the length of the contractile vacuole, moved in the normal fashion, revolving to the left, and moving with the anterior (cut) end in front. The reactions to stimuli in such a small piece are very difficult to determine.

IV. *Paramecium caudatum.* — *Paramecium* is, of course, much less favorable for work of this character than are the infusoria of which an account is given above. This is due in part to the minute size and rapid movements of *Paramecium;* in part to the strong tendency for the cut pieces to disintegrate at once. But, with much patience, satisfactory anterior and posterior halves can be obtained.

1. Anterior half, bearing the oral groove and mouth (Fig. 4, *a*). This moves and reacts almost exactly as does the entire animal,—

revolving to the left, and turning toward the aboral side when stimulated mechanically or chemically.

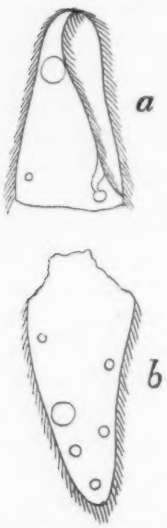

FIG. 4. Paramecium cut transversely. *a*, anterior half, with mouth and oral cilia. *b*, posterior half, without mouth or oral cilia.

2. Posterior half, without oral groove or mouth (Fig. 4, *b*). Much pains was taken to remove the entire oral groove and mouth, so that the posterior piece was usually a little less than one half the body. It was possible to distinguish the aboral side by the presence there of the contractile vacuole. The posterior half swims in a spiral, revolving, like the uninjured animal, to the left, and keeping the aboral side to the outside of the spiral. Its movements are slower than those of the anterior half, and it is much less sensitive, not reacting so readily to stimuli. The reaction to mechanical and chemical stimuli is by jerking backward and turning toward the aboral side, as in the uninjured individual. Thus the presence of the oral groove and oral cilia is not necessary in order that the usual movements and reactions may occur.

3. Middle third of *Paramecium* (anterior and posterior ends removed). — This behaves essentially like the anterior and posterior halves, or the entire animal.

SUMMARY AND CONCLUSIONS.

In all the Ciliata studied, including several of the Hypotricha (*Stylonychia*, *Oxytricha*, etc.), two of the Heterotricha (*Stentor* and *Spirostomum*), and one of the Holotricha (*Paramecium*), the movements and reactions of pieces of the organisms, if these are not too minute or too irregular in form, are essentially similar to those of the entire animal. The pieces swim in a spiral, swerving continually toward a certain side, just as do the entire organisms. They react to chemical and mechanical stimuli by backing and turning toward the same structurally defined side, as do the entire animals. This is true of pieces forming one fourth to

one half the entire animal, whether taken from the anterior end, the posterior end, the right or left side, or the middle of the body. It is true, whether the piece does or does not bear any of the oral or other differentiated cilia.

These results establish the following facts:

1. The turning toward a structurally defined side after stimulation is not produced alone by the oral or peristomal or anterior cilia, or by any particular set of cilia, since it takes place in pieces of the body containing no specially differentiated cilia.

2. The swerving toward a certain side in the spiral swimming is not due alone to any special set of cilia, since it occurs in pieces from any part of the body.

3. The swerving toward a certain side is likewise not due to the geometrical form of the body (as to a groove on one side, such as we find in *Paramecium*), for it continues when the form of the piece is essentially altered (as by the removal of the oral groove in *Paramecium*).

4. The revolution on the long axis in a certain direction, while swimming, is not due to any special set of cilia, nor to any peculiarities of form of the body, since it occurs in pieces from all parts of the body.

5. From the foregoing paragraphs, 1–4, it must be concluded that the revolution on the long axis, the swerving toward a defined side in the spiral course, and the turning toward a structurally defined side when stimulated, are due to the method in which the cilia strike. This peculiar (one-sided) stroke is not limited to any particular set of cilia, but is shared by all the body cilia.

Division of labor is thus not marked, so far as these points are concerned, among the locomotor organs of these animals. As any portion of a crystal is organized like the entire crystal, so in the Ciliata any piece of the body, apparently, is organized so as to move and to react to stimuli in the same manner as does the entire animal.

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NOTES ON THE NATURE OF "ARTIFICIAL PARTHENOGENESIS" IN THE EGG OF PODARKE OBSCURA.

AARON L. TREADWELL.

The results obtained by Lillie[1] on the phenomena accompanying artificial parthenogenesis in *Chætopterus* led me to repeat the experiments, using *Podarke obscura*, with the idea of determining if similar results appear in this genus. I was much interested, also, in the question whether cleavage produced by chemical stimulation bears any resemblance to the normal cleavage; the latter, in this form, having been carefully followed.

That *Podarke* eggs would develop into ciliated embryos in this way was first determined by Dr. A. W. Greeley, to whose courtesy I am indebted for suggestions in the use of his method. This was treatment for one hour with a mixture of 20 c.c. of a 2½ nKCl solution + 80 c.c. of sea water. They were then transferred to sea water, and, after a variable length of time, began to divide. A solution of 22½ c.c. of 2½ nKCl solution + 77½ c.c. of sea water was also used, but gave less satisfactory results than the other. Through lack of material I did not use any other solutions, and experimented only with unfertilized eggs. This leaves my observations very incomplete, but the results thus far obtained seem worth recording.

Controls were kept in each experiment. A few of the control eggs after twelve hours or more in sea water passed into what might be termed, by a superficial observer, a "morula" stage. The cytoplasm breaks up into a great many small globules with no trace of nuclear division, the chromatin lying generally in one globule, which is usually larger than the others. These never develop further, and there was never any trace of nuclear cleavage or ciliated embryos in the control eggs. A similar breaking down into a "morula" occurs in unsuccessful experiments among

[1] F. R. Lillie, Differentiation without Cleavage in the Egg of the Annelid *Chætopterus pergamentaceus*. Archiv f. Entwick. d. Organismen, 14 Bd., 3 and 4 Hft.

the eggs treated with the KCl solution and a few usually appear among the ciliated embryos of a successful experiment.

When laid, the egg of *Podarke* passes into the stage of the first polar spindle, and remains in this condition until fertilized. It is not at all uncommon to find among late cleavage stages eggs which have escaped fertilization, and invariably they show the first polar spindle. Treatment with the KCl solution apparently does not stimulate the egg to form the polar globules, for I have not been able to find any of the latter, and in the one-cell stages found in my preparations the chromatin is either packed into a small, deeply staining mass, or scattered through the cytoplasm. Apparently, though I have not as yet sufficient evidence to demonstrate the accuracy of this conclusion, if the causes operating on the egg are such as to produce the former condition, the further change, if any occurs, is purely cytoplasmic in its character, while if the second condition appears, the egg may

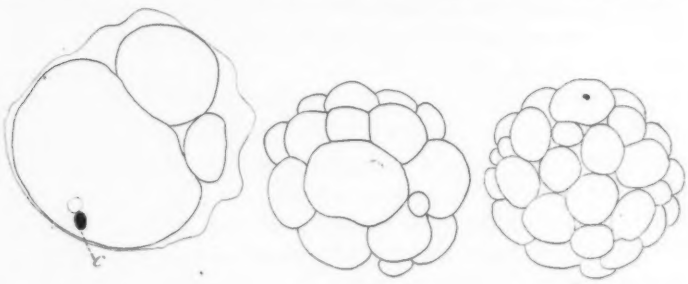

FIG. 1. Division of cytoplasm without nuclear division. *C*, Chromatin. Preserved material.

FIGS. 2 and 3. Like Fig. 1 but showing a more complete division of the cytoplasm. Preserved material.

subsequently divide by a nuclear as well as a cytoplasmic division.

As illustrations of the former case, see Figs. 1 to 3.[1] These were drawn from material preserved two hours and twenty minutes after the transfer to sea water. At this time the eggs were in active

[1] A membrane is present in all of these eggs, but is represented only in Fig. 1. The drawing from preserved material shows a wider space between the egg and the membrane than exists during life.

amœboid movement, though, as the egg membrane is closely applied to the surface of the egg, these movements are not as noticeable as in other animals. They were, however, sufficient in amount to make camera drawings impracticable. The preserved material shows that the cytoplasm is apparently broken up into distinct globules, though from the appearance presented by the living egg I think it altogether probable that they are really in communication with one another, these "globules" being really amœboid processes. As seen in the drawings, these preparations bear a superficial resemblance to a cleaving egg. There is, however, absolutely no resemblance to the normal cleavage, and a study of the stained material shows that the division is one involving only the cytoplasm, the chromatin being massed together in one of the spheres. This sphere is usually, though not always, larger than the others. (In the egg from which Fig. 3 was drawn, the chromatin, except the small bit seen in one of the upper cells, was in a large cell on the side of the egg opposite that from which the drawing was made.)

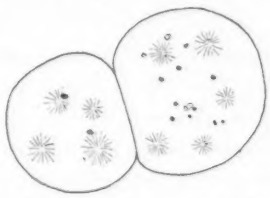

FIG. 4. "Two-cell" stage. The nuclear matter is scattered through the cytoplasm. Preserved material.

In the second of the above-described cases, cleavage involves both nucleus and cytoplasm. In Fig. 4 several asters are plainly to be seen in each cell, which leads to the suggestion that possibly it is upon the presence or absence of these that the differences in nuclear activity are dependent. Division of these eggs is apparently complete. In the two-cell stage the greatest possible variations in size between the two cells appear, the division being sometimes nearly equal, as in Fig. 4 and again very unequal. I have no reason to believe that this division is karyokinetic. It seems rather as if one of the cells is to be regarded as a lobe of protoplasm which contains some of the nuclear material, and may later become completely divided by a membrane from the other cell. Instead of two cells, a trefoil stage may appear.

What I interpret as later developments of the stage just described are shown in Figs. 6 and 7. These divisions are appar-

ently karyokinetic, but in none of them is there the slightest resemblance to the normal cleavage pattern.

Fig. 8 is of especial interest. In about twelve hours after transfer from the salt solution to sea water, there will be found

5 6 7

FIG. 5. Trefoil stage. Preserved material.

FIGS. 6 and 7. Later development of stage shown in Fig. 4. Preserved material.

numbers of swimming embryos, looking much like the half embryo formed by destroying one blastomere of the two-cell stage. It is apparently formed from an embryo like that of Fig. 4 by a development of one cell, while the other remains undivided. Since it seems probable that the latter is to be regarded as a cell with an "extra ovate" attached, rather than a true two-celled

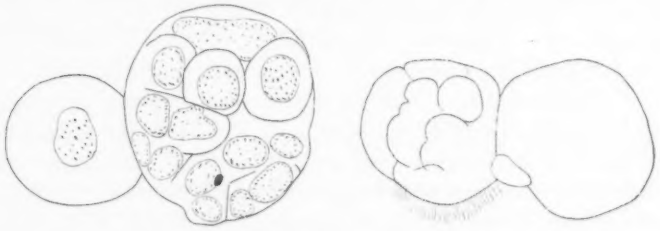

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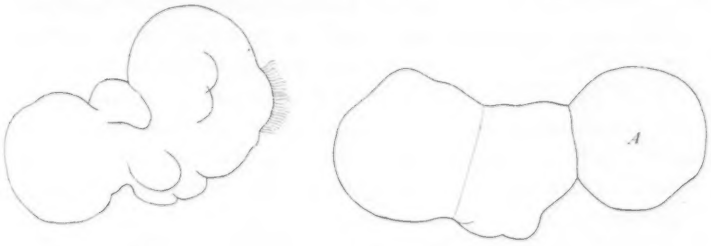
FIG. 8. Embryo produced by development of half of embryo shown in Fig. 4. Preserved material.

FIG. 9. Embryo like that of Fig. 8. Living material.

stage, we might say that here either the extra ovate or its fellow developed, while the other part remained undivided. These divided portions put out cilia, and swim about in the water, carrying the undivided portion with them. Later, they break away

from the undivided portion and swim independently, forming "dwarf embryos." So far as I can discover, their cleavage has no resemblance to the normal. Their abnormal character is also indicated by their nuclei, which have a very characteristic appearance, resembling closely those of abnormal eggs which occasionally appear among a normally fertilized lot. The nuclei are much larger and stain much more intensely than the normal ones. Fig. 9 is from a living egg of this sort, and shows the arrangement of the cilia.

Besides these ciliated embryos are others, showing no indication of cleavage, but with well-developed cilia. Fig. 10 shows



10 11

FIG. 10. Ciliated, unsegmented embryo. Living material.
FIG. 11. Unsegmented embryo formed by fusion. Living material.

one of these where the whole embryo is very much distorted, but with a well-marked tuft of cilia. Staining with aceto-carmine after the above drawing was made, gave no indication of cleavage in this embryo.

Fig. 11 shows a case of fusion of at least two eggs. This was from an embryo slowly rotating around the end *A* as a pivot. I was unable, however, to discover the distribution of the cilia causing this movement.

Fig. 12 shows a still more interesting condition. Here, also, there is apparently a fusion of at least two cells, but there is no trace of cleavage. At *n* is possibly a nucleus. Around one portion of the fused mass is a ring of cilia, occupying very much the position, with respect to the cell, of the prototroch in its relation to the trochophore. Not only are the cilia present, but around the embryo, underneath the ciliated band, is an area free

from the ordinary pigment of the rest of the cell, but containing granules of a faint yellow color, agreeing in this respect exactly with the prototroch band of the normal trochopore. In this embryo there is, then, not merely a differentiation—without cleavage—of cilia, but of the characteristic protoplasm accompanying these cilia—or, in other words, we have here, in the unsegmented embryo, not merely a differentiation of cilia, but a differentiation of *prototroch cilia.* Later, fused masses like those just described are apt to break down, setting free the ciliated fragments, and by the twentieth hour the culture will be swarming with ciliated fragments derived from this source and from the breaking away of the "dwarf" described above. These, of course, have not even a superficial resemblance to normal embryos.

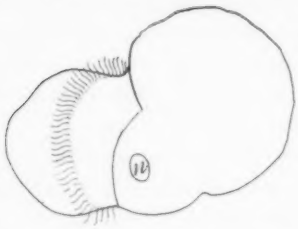


FIG. 12. Unsegmented embryo formed by fusion with well-differentiated band of cilia. Living material.

Fusion phenomena are rare in *Podarke* as compared with *Chætopterus*, the largest fused masses I have found containing not more than six eggs.

SUMMARY.

1. After treatment with 2½ nKCl solution, eggs of *Podarke obscura* exhibit various cleavages and pseudo-cleavages.

2. The pseudo-cleavages involve only the cytoplasm and not the nucleus, and have no resemblance to the normal cleavages.

3. The cleavage involves both nucleus and cytoplasm, and may give rise to a ciliated embryo. The cleavage in this case, however, does not resemble the normal.

4. Ciliated embryos may arise without cleavage. The differentiation may be carried very far in such cases. This is especially noticeable in the character of arrangement of cilia.

5. Fusion of embryos may occur, but in the solution mentioned, much fewer cells unite than in, *e. g.*, *Chætopterus*.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASS., Sept. 5, 1902.

HABIT-FORMATION IN THE GREEN CRAB, CARCINUS GRANULATUS.

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In his excellent paper[1] on the structure of the nervous system of *Carcinus mœnas* Albrecht Bethe describes certain experiments the results of which he believes to justify the conclusion that the green crab does not profit by experience, and therefore does not possess "psychischen Qualitäten."

The following are two of the experiments by which Bethe tested the ability of *Carcinus* to learn. A crab was placed in an aquarium which contained an *Eledone* in the darkest corner. Immediately the crab scurried off into the dark region and was seized by the *Eledone* and drawn under its mantle. The experimenter then interfered by quickly freeing the crab from its enemy and returning it to the opposite end of the basin. Again the crab hurried into the shaded region, was seized, freed and returned to the light. This experiment Bethe says he repeated with one individual five times and with another six times without obtaining any evidence that the crabs were learning to avoid the danger. In another test crabs in a basin were baited with meat, and each time they tried to get the food they were seized and maltreated by the experimenter. Thus an attempt was made to teach them that meat, under certain conditions, meant danger. But in this test also several repetitions of the experiment failed to teach the crabs to avoid the danger.

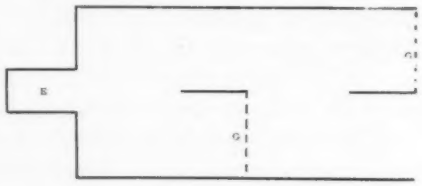
Bethe's tests are unsatisfactory because in each he attempts to inhibit an instinctive action. In the first test the animal naturally sought to hide in the dark; and it would be absurd to expect to modify this instinct by giving the animal five, six or even twenty-five experiences of the disagreeable consequences of going into the neighborhood of the *Eledone*. And in the second test the reflex food-seeking reaction to a particular kind of chemical

[1] Bethe, Albrecht: Das Centralnerven-system von *Carcinus mœnas*. II. Theil., Arch. f. Microscop. Anat., Bd. 51, 1898, S. 447.

stimulation stood in the way of the establishment of new modes of response.

Bethe should not have expected a few experiences to modify or inhibit such fundamentally important reactions as those to light and to food. Almost any reaction can be modified if time enough be devoted to that end, but it is as unlikely that a crab would learn to avoid the dark or to inhibit its food-taking reaction after a half dozen harmful experiences in a certain situation as it is that the grasshopper would learn to inhibit its jumping by a few experiences in a glass box. For the modification of such reactions hundreds, not tens, of experiences are necessary.

In view of the obvious unfairness of Bethe's tests of *Carcinus* I have made a further study of the ability of the American species of *Carcinus*, *C. granulatus*, which is similar to the European form, *C. mœnas*. My effort has been to determine whether



Floor Plan of Experiment Box. Scale one eighth.

the animals can learn (1) a simple labyrinth path to their food, and (2) to avoid the unpleasant experience of being captured in a hand-net and taken from the aquarium.

The crabs were kept in an aquarium about 60 cm. long, 38 cm. wide and 35 cm. deep, over which an experimenting box 40 cm. × 20 × 12 was placed so that its exit passage just touched the surface of the water. The accompanying cut which is the floor plan of the box, shows, in addition to the entrance passage *E*, in which the crab was placed by the operator at the beginning of each experiment, two alleys which were closed by glass plates *G*, *G*. Experiments were made to discover whether the green crab would learn to avoid these blind alleys in its efforts to get back to the water of the aquarium, and if so how quickly it would learn.

The only motive relied upon for the return of the crab to the aquarium from the experimenting box was its desire to get into the water.

For two weeks several crabs were given on an average four trials per day in the labryrinth. At the beginning of the work they frequently wandered in the entrance passage and blind alleys several minutes before they succeeded in escaping, but after twenty or thirty trials most of them were able to escape directly. I was unable to get any satisfactory quantitative expression of the rapidity of learning because of the great variation in strength of the animal's desire to get back to the water; at one time it would move about constantly until it found the exit, at another it would rest quietly in the passages for long periods. In the case of an animal which, for a month, was given two trials per day in the labyrinth, the escape at first took about five minutes, and at the end of the period about ten seconds.

At first the crabs when placed in the box and poked with a stick scurried off into a corner and took a defensive position, whereas, after a few escapes from the box, they made directly for the exit as soon as they were threatened with a stick.

Even fifty experiences, in case of most of the individuals studied, did not result in the formation of a perfect habit.

Further tests of the ability of the green crab to learn were made by placing a wire-screen partition in the middle of the aquarium and allowing the animals to find their way through an opening in the screen to food on the opposite side. Since the crabs tend to follow the edge of such a screen, the opening, 10 cm. x 10, was made in the middle of the screen. It was therefore about 12 cm. distant from the sides, top and bottom of the aquarium.

In this test each crab was given a single experience per day. To provide the animals with a motive for seeking to pass beyond the screen, at the beginning of each trial a piece of fish meat was placed in the corner of the aquarium diagonally opposite to that from which the crab started. The meat having been placed, a crab was put into the aquarium, and a record was kept of its wanderings in search of the food, and of the time occupied in finding it.

The following are the results of ten trials with two animals.

Number 1.		Number 2.	
Trial.	Time in Minutes.	Trial.	Time in Minutes.
1	7	1	23
2	4	2	10
3	$3\frac{1}{2}$	3	$4\frac{1}{2}$
4	4	4	3
5	2	5	$2\frac{1}{4}$
6	1	6	$6\frac{1}{2}$
7	1	7	7
8	$1\frac{1}{2}$	8	$1\frac{1}{4}$
9	1	9	$1\frac{1}{3}$
10	$1\frac{1}{4}$	10	$\frac{1}{2}$

Crab number 1 almost invariably moved directly toward the meat and searched for an opening in the screen along the side of the aquarium nearest the meat, thence it would move to the middle of the screen and find the opening. Number 2 just as uniformly followed the edge of the screen, climbing up one side, along the top, down the other side, and across the bottom ceaselessly until it chanced to get near the opening.

The paths for the first trials were complex and devious, but in the later trials they were almost direct lines to the meat.

There was little evidence of the use of vision in the finding of the opening in the screen. The animals seemed to be directed chiefly by the stimulus from the food.

Additional evidence of the ability of *Carcinus* to profit by experience is furnished by the following observation. It was the habit of the experimenter to catch the animals in a hand-net, take them from the water and use them for various experimental purposes. At first the crabs did not react otherwise to the approach of the experimenter with the net than to other large objects, but after a few days it became much more difficult to capture them for they had learned to avoid the net.